# Effects of preconditioning on myocardial regional contractility during low-flow ischaemia; the possible role of nitric oxide

**Ph.D. Thesis**

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## SUMMARY

It is established that preconditioning protects the myocardium against ischaemic injury (cell necrosis), and severe ventricular arrhythmias that occur during a subsequent ischaemia / reperfusion insult. Apart from the 'classical' ischaemic preconditioning stimulus (i.e. brief coronary artery occlusions), cardiac pacing and physical exercise are also providing preconditioning-like cardioprotection. However, the effect of preconditioning on myocardial contractile function during ischaemia / reperfusion is less characterised.

Therefore, we aimed to examine in an anaesthetized open-chest canine model (1) the changes in regional myocardial contractility during repeated partial coronary artery occlusions and reperfusions, (2) the effects of cardiac pacing and physical exercise, as preconditioning stimuli, on functional changes, occurring during these low-flow ischaemia / reperfusion sequences 24 hours later; and (3) the possible involvement of nitric oxide (NO) in these occlusion / reperfusion-induced contractile changes. This was investigated by the intracoronary administration of L-NAME, a non-selective inhibitor of NO synthase enzymes.

We showed, that repeated low-flow ischaemia / reperfusion cycles (four times for 20 min), produced by an approx. 50% reduction in resting blood flow of the left anterior descending (LAD) coronary artery, resulted in a progressive decrease in segmental shortening within the ischaemic area; the most pronounced reduction in contractility occurred during the third ischaemic insult. In dogs, subjected to cardiac pacing or physical exercise, 24h prior to low-flow ischaemia / reperfusion cycles, the contractility within the ischaemic segment was maximally decreased even during the first ischaemic episode, and no further changes occurred during the subsequent ischaemic / reperfusion interventions.

We have also demonstrated that NO plays a role in this kind of functional (contractile and metabolic) adaptation, since administration of L-NAME abolished these adaptive changes. In the presence of L-NAME, both in the control and paced dogs, the marked decrease in segmental shortening disappeared during the consecutive ischaemic episodes. Further, in the presence of L-NAME the lactate production was significantly increased, indicating a deterioration in metabolic conditions during ischaemia.

Our results show that preconditioning provides protection to the heart during ischaemia by decreasing regional contractile function and thereby the metabolic demands of the myocardium, and that NO plays an important role in this adaptive response. We think that our observations, that physical exercise can induce such an adaptation in the myocardium during ischaemia may have clinical importance.

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## LIST OF PUBLICATIONS

### Full papers

I. Babai L, **Szigeti Zs,** Parratt JR, Végh Á. Delayed cardioprotective effects of exercise in dogs are aminoguanidine sensitive; possible involvement of nitric oxide. *Clinical Science 2002; 102:435-445*

II. **Szigeti Zs,** Simon K, Parratt JR, Végh Á. Effects of delayed preconditioning on myocardial regional contractility during repeated episodes of low-flow ischaemia in anaesthetized dogs; possible role of nitric oxide. *Clinical Science 2004; 106: 201-213*

III. **Szigeti Zs,** Simon K, Papp Gy, Végh Á. Effects of physical exercise on regional contractility during repeated low-flow ischaemic episodes in anaesthetized dogs. *Cardiologia Hungarica 2004; 34:241-248*

### Book chapter

IV. Végh Á, Parratt JR, Babai L, **Szigeti Zs,** Papp JGy. Exercise and delayed preconditioning in the protection of the heart against ventricular arrhythmias: Crucial role of nitric oxide. *Dhalla NS, Takeda N, Singh M, Lukas A (edts). Myocardial ischaemia and preconditioning. Kluwer Academic Publishers, Boston; 2003: pp. 423-443*

### Abstracts

V. **Szigeti Zs,** Simon K, Papp JGy, Parratt JR, Végh Á. Cardiac pacing reduces myocardial contractility during repeated low-flow ischaemia, 24 h later. *J Mol Cell Cardiol 1999; 31: A97*

VI. **Szigeti Zs,** Simon K, Papp JGy, Végh Á. Repeated episodes of low-flow ischaemia decrease myocardial contractility in anaesthetized dogs. *Cardiol Hung 1999; Suppl. 2: 57*

VII. **Szigeti Zs,** Simon K, Papp JGy, Parratt JR, Végh Á. Repeated episodes of low-flow ischaemia reduce contractility and this adaptation can be prevented by intracoronarious L-NAME administration. *Z Kardiol 2000; Suppl. 5*

VIII. **Szigeti Zs,** Simon K, Papp JGy, Végh Á. The effects of the early and the late phases of preconditioning on myocardial contractility during low-flow ischaemia: the role of nitric oxide. *Cardiol Hung 2000; Suppl. 3: 24*

IX. **Szigeti Zs,** Babai L, Simon K, Papp JGy, Parratt JR, Végh Á. The effects of early and late phase preconditioning on myocardial contractility during repeated episodes of low-flow ischaemia: the role of nitric oxide. *Perfusion 2000; 15: Abstract Suppl.*

X. **Szigeti Zs,** Simon K, Papp JGy, Végh Á. Effects of physical exercise on regional myocardial contractile function 24 h prior to repeated episodes of low-flow ischaemia in anaesthetized dogs. *Cardiol Hung 2001; Suppl. 2: 26*

XI. **Szigeti Zs,** Simon K, Papp JGy, Parratt JR, Végh Á. Effects of physical exercise on regional myocardial contractile function during repeated episodes of low-flow ischaemia, 24 hours later. *Acta Clinica Croatica 2001; Suppl. 1: O27*

XII. **Szigeti Zs,** Simon K, Parratt JR, Papp JGy, Végh Á. Effects of physical exercise on regional myocardial contractile function 24 h prior to repeated episodes of low-flow ischaemia in anaesthetized dogs. *Eur Heart J 2001; Abstract Suppl.: P3629*

XIII. **Szigeti Zs,** Simon K, Parratt JR, Papp JGy, Végh Á. Effect of preconditioning on myocardial segmental contractility during repeated low-flow ischaemia in anaesthetized dogs. *J Mol Cell Cardiol 2002;34: A62*

# 1. INTRODUCTION

Ischaemic heart disease is still one of the most serious problems facing modern cardiology and remains one of the leading causes of death in the developed countries. Genetic factors certainly determine the development of this pathological state but the risk is undoubtly increased by other factors, such as smoking, cholesterol rich diet, psychical stress and a sedentary lifestyle. Once the disease has developed, the only possibility is treatment for the rest of life and prevention of further progression and complications; at present we are not aware of a drug or other medical methods which completely obviate this condition.

Myocardial ischaemia, described as an imbalance between energy demand and metabolic substrate supply (including oxygen) of the heart [1], occurs when coronary artery blood flow is partially or fully obstructed. Ischaemia results in cellular hypoxia and this leads to the depletion of high energy stores (glycogen, ATP, phosphocreatine) and accumulation of potentially harmful anaerobic catabolites [2-4]. These may influence the function of contractile elements, and of several proteins as well as membrane ion channels, resulting in complete disturbance of cell homeostasis and, at least, in endangering cell integrity.

## 1.1. New ischaemic syndromes

Partial or complete occlusion of a main coronary artery results in immediate contraction abnormalities within the supplied area [5, 6]. If the occlusion is prolonged (more than 20-30 min), despite restoration of blood flow, the ischaemia and reperfusion-induced alterations become irreversible, and ultimately myocardial cell death may occur [7]. However, this definite conception of the consequences of ischaemia-reperfusion injury has become more complex in the last decades, when the concept of the „new ischaemic syndromes" has been introduced by Opie and his colleagues [8, 9]. They defined three different states of the ischaemic myocardium: myocardial stunning, hibernation, and ischaemic preconditioning. These phenomenons are of great clinical importance and led clinicians to further understanding of the underlying mechanisms during ischaemic challenges of the myocardium.

### *1.1.1. Myocardial stunning*

The phenomenon of the „stunned" myocardium was first described by Braunwald and Kloner in 1982 [10]. They showed in experimental studies that after reperfusion of the ischaemic myocardium, contractility remains suppressed for a longer period (hours to days) even in the lack of necrosis, despite of complete restoration of myocardial blood flow [reviewed in 11 and 12]. Thus, the hallmark of this condition is the presence of a „flow-function mismatch" after successful coronary reperfusion, with normal coronary blood flow but abnormal myocardial contractile function.

Although the clinical diagnosis of myocardial stunning is rather difficult, there are two major clinical situations where it has emerging importance; (i) reperfusion therapy in acute myocardial infarction (thrombolysis and/or coronary angioplasty) and (ii) reperfusion following cardiac surgery or heart transplantation. In general, stunning does not imply a decision-making process, because the myocardium is already reperfused. However, there are some high-risk situations, where stunning can become dangerous and must be treated with the administration of positive inotropic drugs and/or mechanical assistance (i.e. intra-aortic balloon counterpulsation).

The key elements in the pathophysiology of stunning are supposed to be the release of oxygen-derived free radicals during reperfusion, damage of membranes and other intra- and extracellular structures, as well as calcium overload [9, 11, 12].

### *1.1.2. Myocardial hibernation*

This phenomenon has been first recognized under clinical conditions. In 1985 Rahimtoola observed that after successful coronary revascularisation of the ischaemic myocardium, the previous, chronically depressed myocardial contractile function returned to normal or near to normal level (in the lack of previous infarction) [9, 13, 14]. According to the traditional view, the extent of chronic contractile dysfunction would reflect the amount of infarcted tissue. This concept has been changed since in hibernation the preservation of viability is due to the decreasing contractile function rather than to the occurrence of necrosis.

In classical terms the hibernating myocardium is not ischaemic. The typical signs of ischaemia (e.g. lactate production, ATP depletion, creatine kinase leakage) are absent despite the permanent, severe reduction in coronary blood flow [15-17]. However, after several weeks to months a significant loss of contractile elements [18] and other intra- and extracellular

alterations occur [19-21], which are thought to be partialy or completely reversible following revascularisation.

It has been suggested that the hibernating myocardium reduces its function to the extent that blood flow and biochemical function are again in equilibrium. Although there is evidence that segmental contractile function is decreased in proportion to the reduction in regional myocardial blood flow (i.e. „perfusion-contraction matching"; [22]), the precise relationship between these, as well as the underlying mechanisms of the rapid reduction in contractile function remains elusive. Previous studies suggested, that changes in intracellular pH, phosphate concentration [$P_i$] and myocardial NAD/NADH ratio [23], the decrease in myocardial calcium transient and responsiveness [reviewed in 24], and perhaps activation of mitochondrial ATP-dependent potassium channels ($K_{ATP}$) [25] may play a role. Recently, a number of intracellular mediators (e.g. heat shock proteins, oxidative derivates, nitric oxide and several cytokines, such as tumour necrosis factor-alpha {TNF-α}) are thought to contribute to myocardial hibernation, most probably through initiating cellular apoptosis [reviewed in 26 and 27]. However, a clear explanation is still not available.

A returning question in the literature whether chronic hibernation is a form of repetitive, cumulative stunning [11, 28, 29]. Some animal studies have shown, that repeated ischaemic insults may result in cumulative effects on contractility; the duration and the severity of repeated myocardial stunning greatly exceed those induced by a single ischaemic episode [30-33]. Many patients with coronary artery disease experience recurrent episodes of ischaemia within the same myocardial region. These may occur so frequently, that the myocardium remains reversibly depressed for a longer period. It is therefore possible that in certain clinical cases, contractile dysfunction is secondary to repetitive episodes of stunning, rather than results from „pure" hibernation [34]. However, there is some evidence, that the metabolic changes in repetitive stunning and hibernation are different [35]; i.e. opposite to stunning, the regional oxygen consumption in the hibernating myocardium is downregulated [36]. This also confirms different mechanisms, although there is more likely that these two phenomenons exist together in the ischaemic heart [34, 37].

### *1.1.3. Ischaemic preconditioning*

Ischaemic preconditioning was first described by Murry and his colleagues in 1986 [38]. They showed in dogs that four, 5 min occlusion periods of the circumflex branch of the

left coronary artery, rather than resulting in cumulative ischaemic damage, significantly reduced the extent of ischaemic necrosis of a subsequent 40 min coronary occlusion. Preconditioning protects the myocardium against ischaemic damage (i.e. decreases infarct size) [38-40], against ischaemia and reperfusion-induced severe ventricular arrhythmias [41-45] and enhances the postischaemic contractile recovery (reduces myocardial stunning) [46-48]. Preconditioning also improves metabolic conditions of the myocardium during ischaemia [49, 50], decreases myocardial oxygen consumption [51] and protects the endothelium against ischaemia-induced endothelial injury [52]. The protective effects of preconditioning could be proved in all studied species, including humans [53]. Apart from the „classical" ischaemic preconditioning (i.e. repeated, short coronary occlusions; [38]), endogenous protection can be induced by rapid cardiac pacing [45, 54-58], partial occlusion of a coronary artery [59-62], cyclic flow variations [63], alterations in myocardial energy supply-demand balance [64], myocardial stretch [65], and local administration of several substances („pharmacological preconditioning"; [66, 67]), or as recently described, by physical exercise [68-70].

The protective effects of preconditioning appear in two distinct phases. The initial phase occurs immediately after the preconditioning stimulus. This protection is extremely pronounced, but unfortunately transient; it lasts only for minutes or at most 1 to 2 hours. After this the protection disappeares [9, 11, 12, 71]. The protection, however, reappeares 20-24 h later [72] and this phase is known as delayed or second window of protection. Previous results of our group demonstrated that this „second window" of protection against ventricular arrhythmias disappeares about 48 or 72 hours after the cardiac pacing (preconditioning) stimulus [45]. However, if the pacing stimulus is repeated 48 h after the initial preconditioning intervention, the antiarrhythmic protection can be prolonged for further 48 or 72 hours (i.e. 5 days after the initial stimulus) [73].

The mechanism of this marked endogenous protective phenomenon is still not completely understood, but there is strong evidence that endogenous diffusible mediators, released during ischaemia and reperfusion (i.e. preconditioning), such as adenosine, bradykinin, nitric oxide (NO), some prostanoids, endothelin, cathecolamines, oxygen free radicals, and opioid peptides may play a role in both phases of the adaptive response [74]. These mediators, through activation of membrane and/or soluble intracellular receptors, *(i)* initiate rapid intracellular alterations and *(ii)* through activation of various intracellular signalling mechanisms or pathways, lead to indication of *de novo* synthesis of several cardioprotective proteins (such as inducible nitric oxide synthase (iNOS), heat shock proteins, and antioxidant enzymes), which contribute to the delayed phase of protection [75, 76]. The

role of sarcolemmal, and more likely mitochondrial ATP-dependent potassium ($K_{ATP}$) channels, as end effectors in the adaptive process has been described [77, 78].

## 1.2. Relationship between coronary blood flow and myocardial contractile function

As it was mentioned above, under coronary hypoperfusion, without previous ischaemic interventions, myocardial contractile function is decreased in proportion to the reduction in regional blood flow („perfusion-contraction matching"; [22]).

However, there are a number of situations when such a direct connection between coronary flow and contractile function disappeares. For example, in the stunned myocardium, following reperfusion of the ischaemic myocardium, coronary blood flow completely restores, while myocardial contractility remains depressed for a longer period (from hours to weeks) [10]. Similarly, a „perfusion-contraction mismatch" may occur after coronary microembolisation [79-82]. Infusion of microparticles into a main coronary artery results in a transient decrease in coronary and myocardial tissue blood flow, as well as in regional contractility whithin the supplied area, but these flow changes return to normal, while segmental contractile function remains decreased. NO and TNF-α seem to mediate this functional deterioration [82]. Coronary microembolisation is common in the clinical practice; e.g. during distal embolisation of an unstable plaque, or during angioplasty of an atherosclerotic or thrombotic coronary lesion [80].

There are further ischaemic conditions, when „perfusion-contraction mismatch" may develop. For example, Schröder et al. showed in anaesthetized dogs, that the decrease in regional myocardial contractility during two, consecutive episodes of partial occlusion resulting from the same grade of flow reduction was more marked, if a preconditioning stimulus (5 min total occlusion) had been performed between these partial occlusions [83, 84]. Similarly, Minamino et al. [50] observed in dogs that (compared to the non-preconditioned controls), a single 5 min preconditioning stimulus resulted in a more marked depression in segmental contractile function and a less pronounced metabolic deterioration during a subsequent low-flow ischaemic episode. Recently, Marti et al. reported that in anaesthetized dogs, 5 days after a series of preconditioning stimuli (20 times 2 min of total coronary occlusions), the same grade of low-flow ischaema resulted in a more pronounced decrease in segmental shortening [85]. All of these studies indicate that following preconditioning the myocardium more easily adapts to the ischaemic conditions, which is manifested in a reduced

myocardial contractile function and in a better preserved myocardial metabolism during the subsequent same grade of low-flow ischaemia.

### 1.3. Aims of the study

Re-occuring ischaemia is common feature in ischaemic heart disease. In patients with severe coronary artery disease, even the daily physical activity may result in recurrent ischaemia in the area supplied by a stenosed coronary artery [reviewed in 86]. Similarly, enhanced vasoreactivity of the culprit lesion in unstable angina may lead to frequent coronary spasms and repeated ischaemic episodes [87]. Nevertheless, if several ischaemic episodes occur within a short time, the first ischaemic event may act as a preconditioning stimulus and influences the consequences of the following ischaemic insults [88, 89]. At present, our knowledge is incomplete as regard changes in myocardial contractile function and metabolism during repeated ischaemic episodes. Further, we do not know whether preconditioning would modify regional myocardial contractile function during low-flow ischaemia 24 h later and what possible mechanisms are involved in this form of adaptation.

The main objective of the present thesis was to examine in open-chest, anaesthetized dogs, the changes in regional myocardial contractile function during repeated low-flow ischaemia and to determine whether preconditioning, induced by cardiac pacing or by physical exercise influences myocardial segmental contractility during these low-flow ischaemic episodes. To achieve this the followings have been analyzed:

(1) The mechanical and metabolic changes during repeated episodes of low-flow ischaemia and reperfusion;

(2) The effects of pacing-induced preconditioning on mechanical and metabolic conditions during these low-flow ischaemic cycles, commenced 24 h later;

(3) The effects of a single episode of physical exercise, induced 24 h previously, on segmental contractility during low-flow ischaemia;

(4) The possible role of nitric oxide in this cardiac adaptive phenomenon.

# 2. MATERIALS AND METHODS

## 2.1. Experimental animals

Adult mongrel dogs of either sex, weighing in excess of 27 kg and allowed to access to food and water ad libitum a day before until starting the experiments, were used. The dogs were kept under quiet conditions at least one week before the commencement of the experiments and received humane treatment according to Hungarian and European laws, and to local institutional policy.

## 2.2. Pacing procedure

On the day of intervention dogs were lightly anaesthetized with an intravenous injection of sodium pentobarbitone (Phylaxia-Sanofi, Hungary) and allowed to breath spontaneously. A Cordat F4 bipolar pacing electrode was inserted, by way of the right jugular vein, into the right ventricle such as that it made contact with the ventricular endocardium. The correct position of the electrode was controlled by recording the endocardial electrocardiogram. Arterial blood pressure was monitored from the left carotid artery. Dogs were paced, at a rate of 220 beats $min^{-1}$ for four 5 min periods, with 5 min resting intervals between the the pacing stimuli, as described previously [45]. In the sham-operated dogs the pacing electrode was introduced into the right ventricle but the dogs were not paced. The animals were then allowed to recover from the anaesthesia and kept separated under quiet conditions.

## 2.3. Physical exercise procedure

A separate group of dogs was allowed to adapt to laboratory conditions for a week. During this period the animals were transported to the laboratory and made to stand on the treadmill but were not exercised. After one week, dogs were subjected to a total exercise period of 21 min. The exercise protocol is shown in **Figure 1**. The slope and the speed of the treadmill were increased every 3 min reaching the maximum load during the final 3 min period. Heart rate (HR), measured from a chest lead surface electrocardiogram, was recorded

during exercise. The sham-exercised dogs were only standing on the treadmill during the same period. After this procedure the animals were kept separated under quiet conditions.

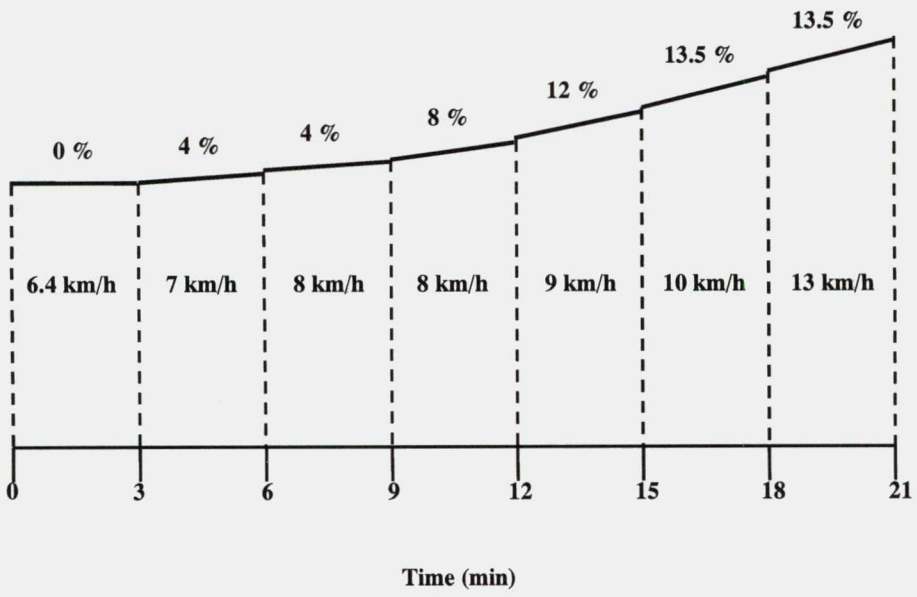


**Figure 1.** *Exercise protocol.* Instrumented dogs were subjected to treadmill exercise for a period of 21 min. Slope and speed of the treadmill were increased every 3 min, started from 6.4 km $h^{-1}$ and 0 % grade. By the end of the exercise period the dogs ran on the treadmill with a speed of 13 km $h^{-1}$ and a slope of 13.5 %.

## 2.4. Surgical interventions

The experimental model is illustrated in **Figure 2**. Twenty-four hours after the preconditioning (pacing or exercise) procedures, dogs were anaesthetized with sodium pentobarbitone (30 mg $kg^{-1}$, i.v.). If necessary, additional doses of anaesthetic (100 μg $kg^{-1}$ $min^{-1}$ i.v.) were infused during the experiment. The dogs were then intubated, and ventilated with room air (Harvard respirator, USA) at a rate and volume sufficient to maintain the physiological level of arterial blood gases and pH. Body temperature was monitored in the mid-oesophagus and maintained, by a heating pad, around 37 ± 0.5 °C. Polyethylene catheters were inserted into the right femoral artery for the measurement of blood pressure; into the left femoral artery to withdraw arterial blood samples; into the left ventricle, by way of the left carotid artery, for the measurement of left ventricular pressure and dP/dt; and into the right femoral vein for drug and anaesthetic administration. The pressure catheters were connected

via transducers (P23XL Statham, Hugo Sachs, Germany) to the System-6 six channel haemodynamic apparatus (Triton Technologies, USA). These traces, together with a standard limb lead electrocardiogram, were recorded on an eight channel chart recorder (Medicor, Hungary) [90].

Thoracotomy was performed in the fifth intercostal space and the anterior descending branch of the left coronary artery (LAD) was prepared. Proximal to the first main diagonal branch a screw-type occluder was placed on the coronary artery. Just proximal to the occluder a Doppler flow-probe (diameter: 2 mm; Triton Technologies, USA) was positioned around the coronary artery to measure coronary blood flow (CBF) velocity (cm $s^{-1}$). The severity of myocardial ischaemia was assessed from changes in the ST-segment, recorded from a hook-formed copper electrode fixed in the endocardium within the ischaemic area [90].

In some dogs, a small side branch of the LAD, near to the occlusion site, was prepared and catheterised. It was used either for the administration of $N^{\omega}$-nitro-L-arginine-methyl-esther (L-NAME; Sigma) or of saline. In some of the dogs the local coronary vein adjacent to the LAD was cannulated and venous blood samples were taken for lactate determination.

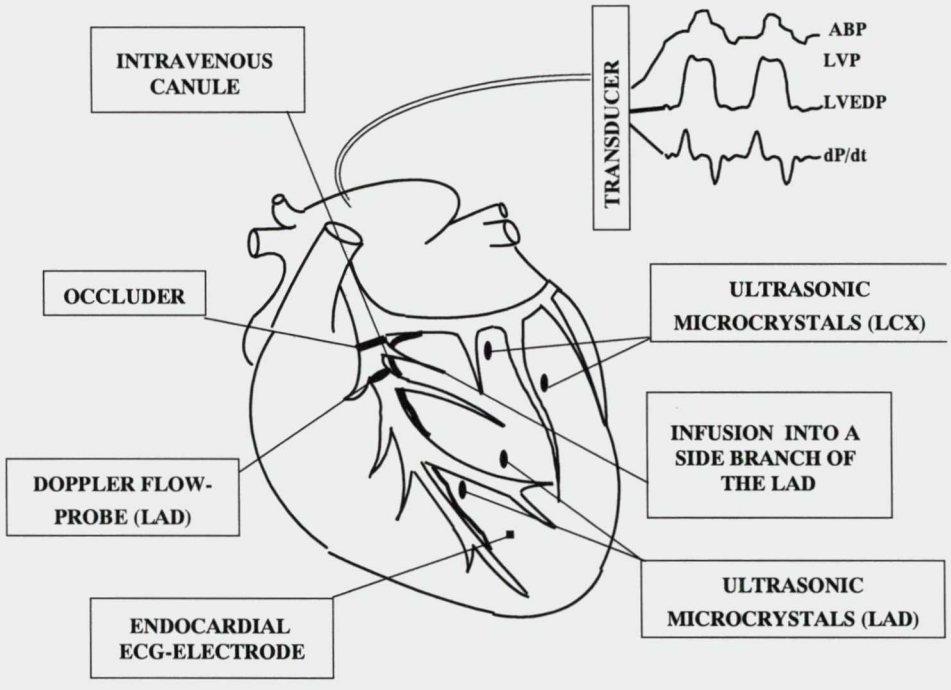


**Figure 2.** *The experimental model* in anaesthetized dogs for the measurements of haemodynamic parameters, coronary blood flow and segmental contractile function, as well as for the registration of changes in endocardial ST-segment elevation.

## 2.5. Measurement of the regional myocardial contractile function

Regional myocardial contractile function was measured by ultrasonic technique as described by Theroux and colleagues [91]. In brief, two pairs of piezoelectric microcrystals were positioned in the endocardium both in areas supplied by the constricted LAD (ischaemic area) and the patent left circumflex (LCX; non-ischaemic area) coronary arteries. The emitter and receiver crystals, within each pair, were placed 8-12 mm apart and orientated along the main axis of contraction. From the contractile curve, end-diastolic segment length (EDL) was measured at the rapid initial upstroke of the left ventricular pressure curve, and the end-systolic segment length (ESL) was determined at the maximum of the negative left ventricular dP/dt curve. Local myocardial contractile function was expressed as percent changes in systolic segmental shortening (%SS) and described by the following equation:

$$\%SS = (EDL - ESL) / EDL \times 100\ (\%)$$

## 2.6. Assessment of myocardial tissue blood flow

In four paced and in four control dogs, changes in myocardial tissue blood flow were measured by means of the 'coloured microspheres' method [92]. Ten million microspheres (15 μm), in one of four different colours, were injected through the left atrium immediately prior to the first episode of ischaemia (baseline) and also at 15 min during the first, third, and fourth occlusion periods (see chapter 2.10. Experimental protocols). Blood samples were collected from the thoracic aorta. At the end of the experiments the hearts were sectioned into vertical slices and endocardial and epicardial samples were taken from both the ischaemic (LAD) and the non-ischaemic (LCX) myocardial regions. Blood and tissue samples were then frozen for further processing. After digestion of the samples with potassium hydroxide, dye was extracted with dimethyl-formamide (DMF, Sigma) and the absorbance was measured via spectrophotometry (HP8453 UV-Vis Spectrophotometer, Hewlett-Packard, USA). Tissue blood flow, expressed in ml $min^{-1}$ $g^{-1}$ was calculated using a software developed in the Department of Pathophysiology, University of Essen, Germany [92].

## 2.7. Lactate determination

In four dogs of each of the paced and the sham-paced groups, blood samples were taken from the local coronary vein adjacent to the LAD and from the right femoral artery,

immediately prior to ischaemia and then again at the end of each of the low-flow ischaemic episodes. The samples were centrifuged (3000 rpm) and the supernatants stored at -18 °C. Lactate extraction ratio (LER) was calculated according to the following equation:

$$LER = ([Lact]_{art} - [Lact]_{ven}) / [Lact]_{art} \times 100\ (\%)$$

where $[Lact]_{art}$ = arterial plasma lactate concentration and $[Lact]_{ven}$ = venous plasma lactate concentration.

### 2.8. Histological examination

At the end of the experiments, in four control and four previously paced dogs, transmural sections were made from the anterior (ischaemic) and from the posterior (non-ischaemic) regions of the ventricular wall. These tissue samples were fixed in 10 % formaldehyde and embedded in paraffin. Differences in tissue structure and glycogen content were examined with light microscopy after staining with haematoxylin and eosin, periodic acid Schiff (PAS) and PAS after amylase pretreatment.

### 2.9. Assessment of the area at risk

At the end of the experiment, the heart was stopped by an overdose of pentobarbitone and by intravenous injection of potassium chloride. The heart was removed and patent blue V dye was injected into the re-occluded LAD coronary artery (except in dogs, where myocardial samples were taken for the assessment of tissue blood flow or for histological examination). The delineated (risk) area was expressed as a percentage of the left ventricular wall together with the interventricular septum.

### 2.10. Experimental protocols

*2.10.1. Protocol for the evaluation of changes induced by repeated ischaemia-reperfusion cycles, and modification of these changes by cardiac pacing. Assessment of the role of nitric oxide*

This is illustrated in **Figure 3**. Four groups of dogs were used. The animals were randomly assigned to control or paced groups. In the control group (**group 1**; n=16) dogs underwent sham-operation and 24h later, after surgical intervention they were subjected to

four episodes of low-flow ischaemia. This was induced by the constriction of the LAD using the screw-type occluder, in order to produce an approximately 45-50% reduction in resting CBF. Each ischaemic episode was lasted for 20 min and followed by a 20 min reperfusion interval. Another group of dogs (**group 2**; n=15) underwent cardiac pacing, as described above, and 24 h later these dogs were also subjected to the similar four ischaemia/reperfusion insults. A further group of paced dogs (**group 3**; n=9) received $N^{\omega}$-nitro-L-arginine-methyl-esther (L-NAME), a non-selective inhibitor of nitric oxide formation through nitric oxide synthase (NOS). This was infused into the small branch of the LAD, in a dose of 10 $\mu g\ kg^{-1}\ min^{-1}$, over a period of 10 min just prior to the first ischaemic insult. In previous studies, this dose of L-NAME attenuated the coronary vasodilator effects of the 20 $ng\ kg^{-1}\ min^{-1}$ dose of bradykinin by 85% [93]. Non-paced, sham-operated dogs (**group 4**; n=10) were also given L-NAME by intracoronary infusion prior to the ischaemia.

In order to evaluate the effectiveness of NOS inhibition by L-NAME, a separated group of dogs (n=6) were given increasing doses of bradykinin (1, 2.5, 5, 10 and 25 $ng\ kg^{-1}$), injected locally into a small side branch of the LAD prior to, immediately after, and again 40 min after the intracoronary administration of L-NAME (10 $\mu g\ kg^{-1}\ min^{-1}$, over 10 min). The vasodilator activity of bradykinin, which depends on the endothelial release of NO, was assessed by the measurement of changes in CBF. From the flow traces dose-response curves were plotted and $ED_{50}$ values were calculated.

*2.10.2. Protocol for the evaluation of the effects of physical exercise on low-flow ischaemia-reperfusion-induced changes, 24 h later*

This is illustrated in **Figure 4**. Two groups of dogs were used. In the exercised group (n=7) dogs underwent physical exercise, as described above, and 24h later they were subjected to two episodes of low-flow ischaemia. This was induced by the constriction of the LAD in order to produce an approximately 45-50% reduction in resting CBF. Each ischaemic episode lasted for 20 min and was followed by a 20 min reperfusion. Another group of dogs (n=9) served as controls, in this group dogs were standing on treadmill without exercise. Twenty-four hours later these animals were also subjected to the same ischaemia/reperfusion insults.

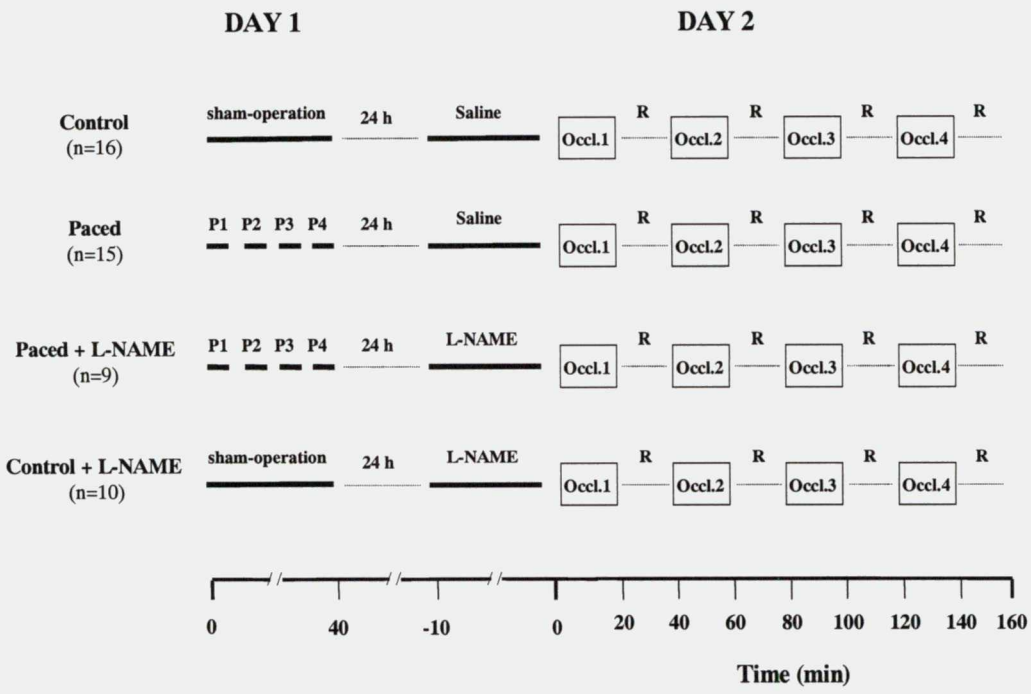


**Figure 3.** *Experimental protocol 1.* On day one dogs underwent either right ventricular pacing or sham-operation. Twenty-four hours later the animals were subjected to four low-flow ischaemia / reperfusion interventions by partial occlusion of the LAD (50% reduction in CBF). Both control and paced dogs were given either saline (i.c. 0.5 ml $min^{-1}$) or L-NAME (10 μg $kg^{-1}$ $min^{-1}$) by intracoronary infusion, 10 min prior to the first ischaemic insult. Abbreviations: Occl. = partial occlusion; R = reperfusion; P = pacing (4x5 min, 220 beats $min^{-1}$); L-NAME = $N^G$-nitro-L-arginine methyl ester.

## 2.11. Statistical evaluation

All data are expressed as means ± s.e.m. and statistical analysis was performed by repeated measures two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. A one-way ANOVA was undertaken to determine whether or not there were significant haemodynamic changes for drug treatment. Differences were considered significant if $P<0.05$.

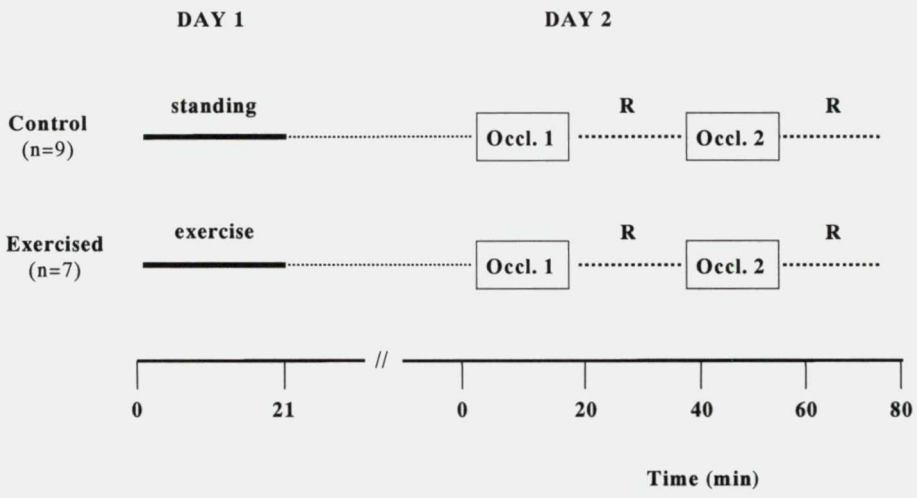


**Figure 4.** *Experimental protocol 2.* On day one dogs were exercised or stand on the treadmill and then 24h later, were subjected to two episodes of low-flow ischaemia and reperfusion insult by partially occluding the LAD coronary artery (50% reduction in CBF).

# 3. RESULTS

## 3.1. Effects of repeated low-flow ischaemia and reperfusion insults (control group)

### *3.1.1. Haemodynamic changes during repeated partial occlusions of the LAD*

There were no significant differences in any of the haemodynamic parameters, measured either at baseline or during the repeated occlusion-reperfusion insults. The data for the four groups are given in **Table 1**. Partial occlusion of the LAD resulted in slight reductions in positive and negative LV $dP/dt_{max}$ and a moderate increase in LVEDP. These alterations were rapidly returned to normal following reperfusion of the ischaemic myocardium.

| | **Baseline** | **O 1** | **R 1** | **O 2** | **R 2** | **O 3** | **R 3** | **O 4** | **R 4** |
|---|---|---|---|---|---|---|---|---|---|
| **1. Control group** | | | | | | | | | |
| Heart rate ($min^{-1}$) | 101±3 | 100±3 | 99±3 | 99±3 | 97±3 | 96±4 | 95±3 | 95±4 | 93±4 |
| SABP (mmHg) | 122±3 | 119±3 | 117±3 | 116±2 | 117±2 | 113±3* | 117±2 | 113±3 | 116±2 |
| DABP (mmHg) | 83±3 | 80±3† | 80±2 | 78±3 | 79±3 | 77±3 | 80±3 | 77±3 | 79±2 |
| MABP (mmHg) | 96±3 | 93±3 | 93±2 | 91±2 | 91±3 | 89±2 | 92±3 | 89±3 | 92±2 |
| LVSP (mmHg) | 119±3 | 114±3 | 114±3 | 110±2* | 112±2 | 108±3* | 112±2 | 107±3* | 111±2 |
| LVEDP (mmHg) | 7.3±0.8 | 9.8±0.7* | 7.6±0.7 | 9.7±0.8 | 7.8±0.8 | 9.4±0.7 | 7.2±0.8 | 9.1±0.9 | 7.2±0.8 |
| $+dP/dt_{max}$ (mmHg $s^{-1}$) | 2109±110 | 2012±86 | 2097±104 | 1914±80* | 2011±100 | 1808±80* | 1996±110 | 1834±79* | 1988±92 |
| $-dP/dt_{max}$ (mmHg $s^{-1}$) | 2399±195 | 2146±166* | 2288±186 | 2088±190* | 2287±199 | 1987±195* | 2234±193 | 1978±184* | 2190±191* |
| **2. Paced group** | | | | | | | | | |
| Heart rate ($min^{-1}$) | 107±5 | 108±4 | 108±5 | 108±4 | 105±4 | 107±4 | 104±4 | 102±4 | 102±4 |
| SABP (mmHg) | 116±3† | 113±4 | 115±3 | 115±3 | 115±2 | 113±2 | 115±2 | 111±3 | 112±2 |
| DABP (mmHg) | 77±2† | 76±3† | 76±2† | 77±2 | 78±3 | 77±3 | 78±3 | 75±3 | 77±3 |
| MABP (mmHg) | 90±2† | 88±3† | 89±2† | 90±2 | 91±2 | 89±3 | 90±2 | 87±3 | 89±36 |
| LVSP (mmHg) | 111±3† | 108±4 | 110±3 | 109±3 | 111±2 | 108±2 | 110±2 | 106±2 | 108±3 |
| LVEDP (mmHg) | 7.0±0.6 | 9.7±0.8* | 7.7±0.7 | 10.2±1.0* | 7.7±0.9 | 10.0±0.9* | 7.5±0.9 | 9.5±1.0 | 7.5±0.9 |
| $+dP/dt_{max}$ (mmHg $s^{-1}$) | 2154±119 | 1956±105* | 2089±102 | 1934±89* | 2122±103 | 1941±96 | 2156±104 | 1884±83* | 2073±92 |
| $-dP/dt_{max}$ (mmHg $s^{-1}$) | 2319±134 | 2006±120* | 2202±124* | 2031±120* | 2174±111 | 1965±108* | 2124±107 | 1861±106* | 2072±105* |
| **3. Paced + L-NAME** | | | | | | | | | |
| Heart rate ($min^{-1}$) | 104±6 | 105±5 | 103±6 | 101±6 | 100±7 | 99±7 | 99±7 | 101±7 | 100±8 |
| SABP (mmHg) | 117±1 | 114±3 | 117±3 | 112±3 | 114±3 | 109±3 | 112±3 | 109±4 | 112±4 |
| DABP (mmHg) | 77±3 | 73±3 | 77±3 | 73±3 | 75±4 | 71±3 | 73±3 | 72±3 | 74±4 |
| MABP (mmHg) | 91±2 | 87±3 | 91±3 | 86±3 | 88±4 | 84±3 | 86±3 | 84±3 | 87±4 |
| LVSP (mmHg) | 112±2 | 109±2 | 112±3 | 107±2 | 109±3 | 104±3 | 106±4 | 104±3 | 107±4 |
| LVEDP (mmHg) | 8.1±1.2 | 11.9±1.4* | 9.2±1.2 | 11.4±1.3* | 9.2±1.3 | 12.2±1.4* | 8.3±1.4 | 11.4±1.5* | 8.9±1.1 |
| $+dP/dt_{max}$ (mmHg $s^{-1}$) | 1916±94 | 1733±99 | 1902±92 | 1771±100 | 1911±95 | 1736±112 | 1909±113 | 1716±112 | 1921±112 |
| $-dP/dt_{max}$ (mmHg $s^{-1}$) | 2345±226 | 2119±224 | 2275±220 | 2095±237 | 2256±241 | 2063±240 | 2204±251 | 2013±228* | 2171±233 |
| **4. Control+L-NAME** | | | | | | | | | |
| Heart rate ($min^{-1}$) | 98±4 | 98±4 | 97±4 | 94±4 | 93±4 | 90±4 | 88±3* | 87±3* | 88±4 |
| SABP (mmHg) | 129±4 | 125±4 | 123±4 | 116±4* | 117±4* | 113±4* | 114±3* | 114±3* | 116±3* |
| DABP (mmHg) | 95±5 | 95±5 | 92±5 | 88±5 | 88±4 | 85±4 | 86±4 | 85±4 | 86±4 |
| MABP (mmHg) | 106±4 | 105±4 | 102±4 | 98±5* | 98±4 | 94±4* | 95±3* | 95±4 | 96±3 |
| LVSP (mmHg) | 123±3 | 119±4 | 118±4 | 112±4* | 113±4 | 108±4* | 110±3* | 109±3* | 111±3 |
| LVEDP (mmHg) | 8.3±0.9 | 11.0±1.5* | 8.3±1.6 | 10.5±1.5 | 7.8±1.3 | 10.0±1.2 | 8.3±0.8 | 10.0±1.0 | 8.3±0.8 |
| $+dP/dt_{max}$ (mmHg $s^{-1}$) | 1831±140 | 1719±129 | 1712±124 | 1504±101*† | 1620±114* | 1502±92* | 1603±105* | 1549±106* | 1616±108 |
| $-dP/dt_{max}$ (mmHg $s^{-1}$) | 2166±225 | 1946±193 | 2078±225 | 1769±170* | 1895±175* | 1619±133* | 1823±190* | 1651±159* | 1780±195* |

**Table 1.** *Haemodynamic changes during repeated ischaemia/reperfusion insults*. Data were measured at the end of each intervention (20 min occlusion and reperfusion) and given as means ± s.e.m. *P<0.05 vs. baseline; †P<0.05 vs. control. O = low-flow ischaemia; R = reperfusion; SABP, DABP, MABP = systolic, diastolic, mean arterial blood pressure; LVSP = left ventricular systolic pressure; LVEDP = left ventricular end-diastolic pressure.

*3.1.2. Changes in coronary blood flow and coronary vascular resistance following repeated ischaemia-reperfusion cycles*

In all experiments we attempted to reduce coronary blood flow of the LAD to achieve an approximate 50% reduction of the baseline, resting blood flow. When the ischaemic myocardium was reperfused, a marked (post-occlusion) hyperaemia occurred, with an about three fold increase in CBF in controls. This hyperaemia was similar in magnitude following each of the four constriction periods. The results for partial occlusions 1 to 4 are given in **Table 2**.

Changes in coronary vascular resistance (CVR) mirrored the changes in flow; it raised significantly from the initial value during the first partial occlusion, then decreased again markedly under the baseline following reperfusion. Changes induced by the subsequent occlusion / reperfusion insults were similar to that of the first one (**Table 2**).

*3.1.3. Effects of repeated occlusion-reperfusion cycles on regional myocardial contractile function*

**Table 3** summarises the changes in EDL, ESL and segmental shortening (%SS) during partial coronary artery occlusions and reperfusions within the ischaemic (LAD) region, whereas **Figure 5** shows the time-course of relative changes in %SS during the four ischaemia/reperfusion cycles in the ischaemic and normal myocardial regions. (This is expressed as a percentage of values measured at baseline).

In control dogs, the first partial occlusion of the LAD (occlusion 1) resulted in an immediate, 58 ± 7 % reduction in %SS. This reduction was somewhat less pronounced (40 ± 6 %) by the end of the 20 min ischaemic episode, indicating that the pre-existing collaterals were opened ant that this resulted in a slight improvement in contractility whithin the ischaemic area during the first ischaemic period. The following (2 to 4) ischaemic episodes resulted in a more pronounced reduction in %SS (59 ± 6, 68 ± 6 % and 70 ± 6%, respectively; $P<0.05$; **Figure 5**). When the myocardium was reperfused, a transient increase in contractile function (of between 10 and 30 %) occurred, which lasted for about 5 min; then %SS was returned near to the initial value. Post-ischaemic myocardial dysfunction (stunning), which was slight (less than 10 % reduction in %SS), developed only after the release of the second

partial occlusion. **Figure 5** also shows that a marked, although statistically not significant „compensatory increase" in segmental contractility (about 15%) occurred within the non-ischaemic (LCX) region when the LAD was repeatedly constricted.

| | 1. Control | 2. Paced | 3. Paced + L-NAME | 4. Control + L-NAME |
|---|---|---|---|---|
| **Baseline** | | | | |
| CBF (cm $sec^{-1}$) | 38.5±2.1 | 36.9±2.6 | 37.4±3.6 | 32.8±2.7 |
| CVR (mmHg $cm^{-1}$ sec) | 2.27±0.17 | 2.28±0.23 | 2.30±0.31 | 2.63±0.24 |
| **Occlusion 1** | | | | |
| CBF (cm $sec^{-1}$) (20') | 21.4±1.1* | 20.1±1.5* | 20.2±1.9* | 17.7±1.5* |
| CBF (% of Baseline) | 55.6±0.8* | 54.5±1.1* | 54.0±2.6* | 54.0±2.8* |
| CVR (mmHg $cm^{-1}$ sec) | 3.94±0.28* | 4.11±0.40* | 4.06±0.60* | 5.78±0.62*† |
| **Reperfusion 1** | | | | |
| CBF (cm $sec^{-1}$) (peak) | 118.0±14.2* | 136.7±17.9* | 119.3±13.9* | 73.8±9.4*† |
| CVR (mmHg $cm^{-1}$ sec) | 0.87±0.13* | 0.65±0.12* | 0.70±0.11* | 1.67±0.37* |
| CBF(cm $s^{-1}$) (pre-occl.) | 37.0±2.6 | 36.7±2.8 | 38.0±3.7 | 32.8±3.1 |
| CVR (mmHg $cm^{-1}$ sec) | 2.36±0.20 | 2.21±0.20 | 2.26±0.32 | 3.07±0.36 |
| **Occlusion 2** | | | | |
| CBF (cm $s^{-1}$) (20') | 20.0±1.3* | 20.3±1.5* | 20.6±2.1* | 17.6±1.6* |
| CBF (% of pre-occl.) | 54.1±0.8* | 55.3±0.8* | 54.2±0.7* | 53.7±0.6* |
| CVR (mmHg $cm^{-1}$ sec) | 4.20±0.35* | 4.12±0.35* | 3.98±0.57* | 5.56±0.73* |
| **Reperfusion 2** | | | | |
| CBF(cm $s^{-1}$) (peak) | 110.5±12.6* | 130.8±18.3* | 102.4±14.2* | 79.5±10.6*† |
| CVR (mmHg $cm^{-1}$ sec) | 0.92±0.16* | 0.68±0.1* | 0.83±0.11* | 1.46±0.32* |
| CBF (cm $s^{-1}$) (pre-occl.) | 37.9±3.3 | 38.5±2.9 | 39.7±3.3 | 34.8±4.6 |
| CVR (mmHg $cm^{-1}$ sec) | 2.38±0.25 | 2.19±0.18 | 2.19±0.32 | 2.93±0.38 |
| **Occlusion 3** | | | | |
| CBF (cm $s^{-1}$) (20') | 20.6±1.8* | 20.9±1.5* | 20.4±1.9* | 18.6±2.4* |
| CBF (% of pre-occl.) | 54.3±0.9* | 54.3±1.0* | 51.4±1.1* | 53.4±0.6* |
| CVR (mmHg $cm^{-1}$ sec) | 4.26±0.44* | 4.00±0.34* | 3.90±0.58* | 5.26±0.70* |
| **Reperfusion 3** | | | | |
| CBF (cm $s^{-1}$) (peak) | 113.2±13.8* | 118.0±20.0* | 116.4±18.8* | 78.7±11.3*† |
| CVR (mmHg $cm^{-1}$ sec) | 0.85±0.11* | 0.56±0.04* | 0.74±0.11* | 1.46±0.32* |
| CBF (cm $s^{-1}$) (pre-occl.) | 37.9±3.6 | 40.1±2.5 | 39.9±3.5 | 37.2±5.7 |
| CVR (mmHg $cm^{-1}$ sec) | 2.40±0.28 | 2.17±0.22 | 1.93±0.20 | 2.80±0.42 |
| **Occlusion 4** | | | | |
| CBF (cm $s^{-1}$) (20') | 20.9±1.8* | 21.6±1.7* | 21.4±1.7* | 19.7±2.8* |
| CBF (% of pre-occl.) | 55.1±0.7* | 53.9±1.0* | 53.6±0.8* | 53.0±1.0* |
| CVR (mmHg $cm^{-1}$ sec) | 4.23±0.48* | 3.94±0.47* | 3.60±0.42* | 5.15±0.75* |
| **Reperfusion 4** | | | | |
| CBF (cm $s^{-1}$) (peak) | 117.0±17.2* | 123.1±22.6* | 115.1±14.6* | 83.2±11.4*† |
| CVR (mmHg $cm^{-1}$ sec) | 0.88±0.12* | 0.54±0.05* | 0.69±0.07* | 1.32±0.27* |
| CBF (cm $s^{-1}$) (20 min.) | 38.1±3.4 | 37.2±4.0 | 41.1±3.5 | 37.6±5.4 |
| CVR (mmHg $cm^{-1}$ sec) | 2.38±0.25 | 2.12±0.24 | 1.94±0.23 | 2.71±0.38 |

**Table 2.** *Changes in coronary blood flow (CBF) and in coronary vascular resistance (CVR)* during repeated partial occlusions and reperfusions of the left anterior descending coronary artery. Data are given as means ± s.e.m., and measured at baseline and at the 20 min of the occlusion (20'). Flow values were also determined immediately after the re-opening of the coronary artery (peak) and at the end of the 20 min of reperfusion (pre-occl.). *P<0.05 vs. baseline; †P<0.05 vs. control group.

| | 1. Control | 2. Paced | 3. Paced + L-NAME | 4. Control + L-NAME |
|---|---|---|---|---|
| **Baseline** | | | | |
| EDL (mm) | 10.02 ± 0.01 | 10.01 ± 0.02 | 9.99 ± 0.02 | 10.12 ± 0.05$^{\sigma}$ |
| ESL (mm) | 8.73 ± 0.09 | 8.73 ± 0.07 | 8.62 ± 0.12 | 8.82 ± 0.15 |
| %SS | 12.67 ± 0.10 | 12.74 ± 0.71 | 13.68 ± 1.11 | 12.88 ± 1.21 |
| **Occlusion 1** | | | | |
| EDL (mm) | 10.60 ± 0.25$^{*}$ | 10.84 ± 0.17$^{*}$ | 10.67 ± 0.13 | 10.57 ± 0.17 |
| ESL (mm) | 9.81 ± 0.30$^{*}$ | 10.39 ± 0.16$^{*}$ | 9.91 ± 0.18$^{*}$ | 9.70 ± 0.26 |
| %SS | 7.67 ± 1.10 | 4.17 ± 0.63$^{\sigma}$ | 7.21 ± 0.76$^{\bullet}$ | 8.27 ± 1.33$^{\bullet}$ |
| **Reperfusion 1** | | | | |
| EDL (mm) | 10.21 ± 0.15 | 10.29 ± 0.14 | 10.26 ± 0.05 | 10.23 ± 0.12 |
| ESL (mm) | 8.99 ± 0.17 | 9.04 ± 0.12 | 8.93 ± 0.13 | 8.95 ± 0.17 |
| %SS | 11.98 ± 1.02 | 12.12 ± 0.71 | 12.99 ± 1.12 | 12.58 ± 1.19 |
| **Occlusion 2** | | | | |
| EDL (mm) | 10.78 ± 0.23$^{*}$ | 10.91 ± 0.21$^{*}$ | 10.49 ± 0.12 | 10.38 ± 0.18 |
| ESL (mm) | 10.23 ± 0.27$^{*}$ | 10.46 ± 0.21$^{*}$ | 9.74 ± 0.17$^{*}$ | 9.56 ± 0.29 |
| %SS | 5.29 ± 0.97$^{\blacksquare}$ | 4.10 ± 0.61 | 7.19 ± 0.78$^{\bullet}$ | 8.09 ± 1.57 |
| **Reperfusion 2** | | | | |
| EDL (mm) | 10.23 ± 0.15 | 10.51 ± 0.20 | 10.12 ± 0.04 | 10.13 ± 0.14 |
| ESL (mm) | 9.04 ± 0.15 | 9.27 ± 0.18$^{*}$ | 8.80 ± 0.13 | 8.91 ± 0.17 |
| %SS | 11.64 ± 1.0 | 11.77 ± 0.75 | 13.05 ± 1.04 | 12.06 ± 1.24 |
| **Occlusion 3** | | | | |
| EDL (mm) | 10.75 ± 0.22$^{*}$ | 10.89 ± 0.22$^{*}$ | 10.45 ± 0.12 | 10.20 ± 0.19 |
| ESL (mm) | 10.33 ± 0.27$^{*}$ | 10.43 ± 0.23$^{*}$ | 9.69 ± 0.19 | 9.35 ± 0.27 |
| %SS | 4.00 ± 0.80$^{\blacksquare\circ}$ | 4.23 ± 0.62 | 7.33 ± 1.02$^{\sigma\bullet}$ | 8.46 ± 1.47$^{\sigma}$ |
| **Reperfusion 3** | | | | |
| EDL (mm) | 10.26 ± 0.14 | 10.30 ± 0.15 | 10.10 ± 0.07 | 10.04 ± 0.15 |
| ESL (mm) | 9.08 ± 0.14 | 9.10 ± 0.16 | 8.80 ± 0.15 | 8.78 ± 0.17 |
| %SS | 11.51±0.95 | 11.70±0.74 | 12.91±1.23 | 12.50±1.22 |
| **Occlusion 4** | | | | |
| EDL (mm) | 10.66 ± 0.25$^{*}$ | 10.75 ± 0.20$^{*}$ | 10.48 ± 0.15 | 10.39 ± 0.21 |
| ESL (mm) | 10.28 ± 0.28$^{*}$ | 10.28 ± 0.21$^{*}$ | 9.70 ± 0.22 | 9.59 ± 0.32 |
| %SS | 3.74 ± 0.75$^{\blacksquare\circ}$ | 4.34 ± 0.66 | 7.49 ± 0.93$^{\sigma\bullet}$ | 7.86 ± 1.66$^{\sigma}$ |
| **Reperfusion 4** | | | | |
| EDL (mm) | 10.20 ± 0.15 | 10.24 ± 0.16 | 10.07 ± 0.09 | 10.06 ± 0.14 |
| ESL (mm) | 9.00 ± 0.16 | 9.01 ± 0.15 | 8.74 ± 0.14 | 8.78 ± 0.16 |
| %SS | 11.79 ± 0.89 | 11.92 ± 0.68 | 13.24 ± 1.20 | 12.72 ± 1.19 |

**Table 3.** *Changes in myocardial end-systolic (ESL) and end-diastolic lengths (EDL), and in segmental shortening (%SS)* within the ischaemic area during repeated partial occlusions and reperfusions of the LAD. Data are expressed as means ± s.e.m., measured at the end (20 min) of each occlusion and reperfusion cycle. $^{*}P < 0.05$ vs. baseline; $^{\sigma}P < 0.05$ vs. control group, $^{\bullet}P < 0.05$ vs. paced group; $^{\blacksquare}P < 0.05$ vs occlusion 1; $^{\circ}P < 0.05$ vs. occlusion 2.

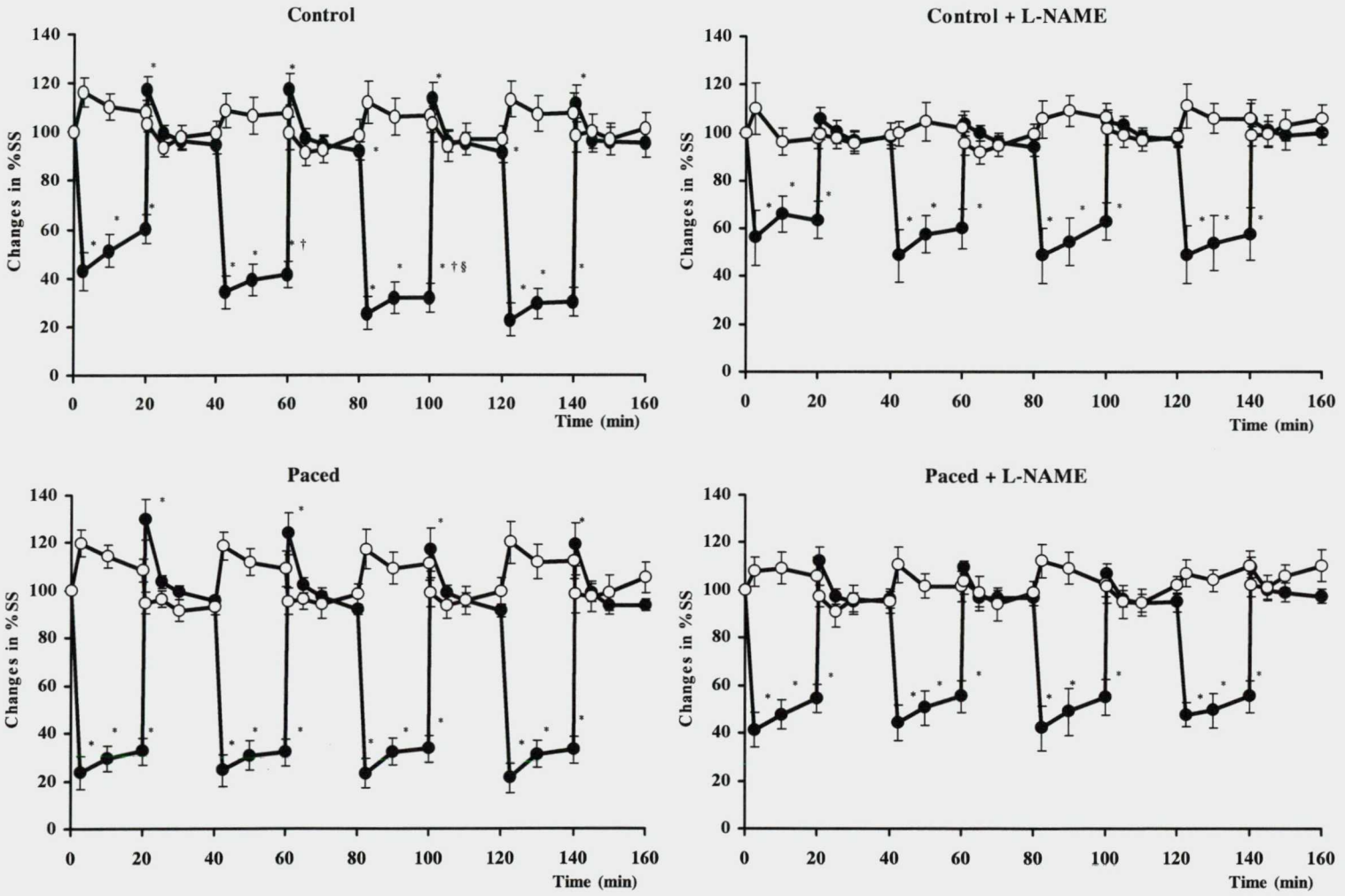


**Figure 5.** *Changes in segmental shortening* within the ischaemic area (supplied by the LAD; filled circles) and non-ischaemic area (supplied by the LCX; open circles) during four consecutive low-flow ischaemia and reperfusion insults in the control and 24 h previously paced dogs, without or with L-NAME pretreatment before ischaemia (baseline value = 100%). Values are means ± s.e.m. $^{*}P < 0.05$ vs. baseline; $^{\dagger}P < 0.05$ vs. 20 min; $^{\S}P < 0.05$ vs. 60 min.

## 3.2. Influences of cardiac pacing and physical exercise commenced prior to the repeated low-flow ischaemia / reperfusion insults

### *3.2.1. Effects of cardiac pacing on heart rate and blood pressure*

Right ventricular pacing at a rate of 220 beats $min^{-1}$, which represents an approximately 81 ± 4 beats $min^{-1}$ increase in HR from the resting value ($P<0.05$), resulted in immediate reductions in systolic arterial blood pressure (of around 49 ± 5 mmHg; $P<0.05$). There was also a slight, transient elevation in the ST-segment (mean of 2.5 ± 0.5 mV; $P<0.05$), recorded from the pacing electrode immediately after cessation of pacing.

### *3.2.2. Effects of physical exercise on heart rate*

Exercise resulted in marked increase in heart rate (from 122±4 to 192±15 beats $min^{-1}$; $P<0.05$) within 1 min of the commencement of exercise which was slightly further increased (to 216±13 beats $min^{-1}$ by the end of the exercise period) **(Figure 6)**. After cessation of exercise, the heart rate returned to resting value within approximately 10 min.

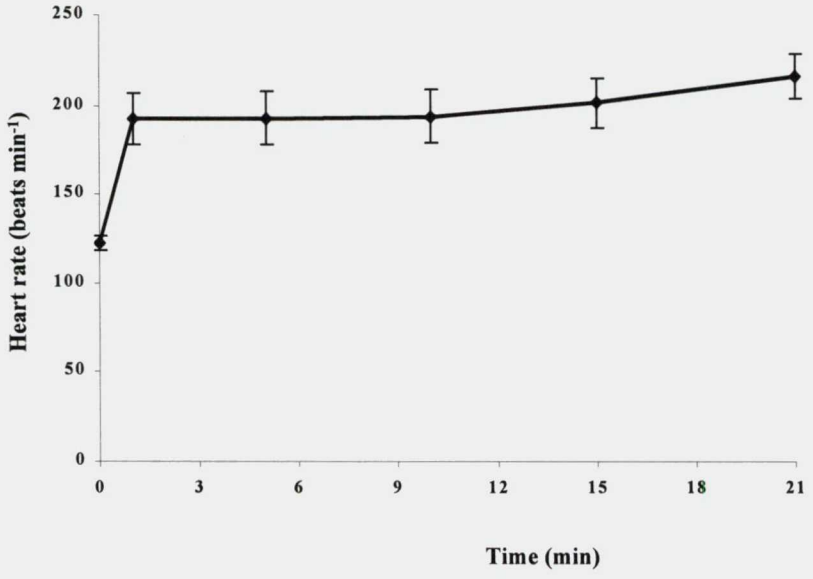


**Figure 6.** *Changes in heart rate during the 21 min period of exercise.* Values are means ± s.e.m. *$P<0.05$ compared to baseline.

*3.2.3. Haemodynamic changes during repeated partial occlusions of the LAD in paced and exercised dogs*

Similarly to the control group, repeated ischaemia / reperfusion cycles resulted in no significant differences in any of the haemodynamic parameters in the paced (**Table 1**) and in the exercised dogs (**Table 4**) 24 h later. There were only slight, not significant decreases in heart rate, arterial blood pressure, positive and negative dP/dtmax and an increase in LVEDP during these ischaemic periods.

| **Control** | HR | MABP | $+dP/dt_{max}$ | $-dP/dt_{max}$ | LVEDP |
|---|---|---|---|---|---|
| Base | 105±5 | 96±3 | 1996±144 | 1999±130 | 7.2±1.4 |
| O 1 | 104±4 | 93±3 | 1926±123 | 1813±110 | 9.2±0.9 |
| R 1 | 103±4 | 93±2 | 1995±147 | 1966±142 | 7.5±1.1 |
| O 2 | 102±4 | 91±3 | 1826±116 | 1698±102 | 9.4±1.1 |
| R 2 | 101±5 | 91±3 | 1983±144 | 1967±141 | 8.1±1.0 |
| **Exercised** | HR | MABP | $+dP/dt_{max}$ | $-dP/dt_{max}$ | LVEDP |
| Base | 109±6 | 102±4 | 2270±152 | 2374±152 | 7.5±1.4 |
| O 1 | 108±6 | 101±4 | 2026±78 | 2232±144 | 10.0±1.7 |
| R 1 | 104±6 | 99±4 | 2158±130 | 2319±153 | 7.1±1.6 |
| O 2 | 103±6 | 100±4 | 1980±98 | 2184±121 | 10.0±2.0 |
| R 2 | 102±6 | 101±4 | 2202±118 | 2380±153 | 7.5±1.8 |

**Table 4.** *Haemodynamic changes during repeated ischaemia / reperfusion insults in control and exercised dogs.* Data were measured at the end of each intervention (20 min of occlusion and reperfusion) and given as means ± s.e.m. O = partial occlusion; R = reperfusion; HR = heart rate (beats $min^{-1}$); MABP = mean arterial blood pressure; dP/dt (Hgmm $sec^{-1}$); LVEDP = left ventricular end-diastolic pressure.

*3.2.4. Changes in coronary blood flow and coronary vascular resistance following repeated ischaemia-reperfusion cycles in paced and exercised dogs*

The LAD coronary artery was constricted in order to achieve an approximately 50 % reduction in resting blood flow. When the artery was reopened, post-occlusion hyperaemia was somewhat more marked both in the paced (4 fold increase in CBF vs. 3 fold in controls; $P<0.05$) and in the exercised groups (about 3.2 fold increase in CBF vs. 2.8 fold in its own controls; ns) than in the control, non-paced and not exercised dogs. This hyperaemic reaction was similar during each of the reperfusion periods. The coronary flow and resistance results for the paced dogs are given in **Table 2** and for the exercised group in **Table 5**. Compared to the controls, there were no marked differences between groups, and changes induced by the

consecutive occlusions / reperfusions were similar to those that occurred during and after the first occlusion.

| | Base | Occl.1 | R 1 min | R 20 min | Occl. 2 | R 1 min | R 20 min |
|---|---|---|---|---|---|---|---|
| **Control** | | | | | | | |
| CBF | 42.7±2.6 | 23.6±1.3* | 120.3±23.6* | 40.8±3.2 | 22.2±1.7* | 115.7±21.3* | 40.9±4.4 |
| CVR | 2.24±0.18 | 4.06±0.26* | 0.78±0.12* | 2.28±0.19 | 4.1±0.28* | 0.78±0.16* | 2.24±0.22 |
| **Exercised** | | | | | | | |
| CBF | 43.9±3.1 | 23.4±1.5* | 142.1±21.2* | 40.2±2.8 | 21.6±1.5* | 140.0±18.6* | 40.3±3.3 |
| CVR | 2.33±0.22 | 4.31±0.28* | 0.73±0.14* | 2.45±0.2 | 4.64±0.32* | 0.72±0.18* | 2.52±0.24 |

**Table 5.** *Changes in coronary blood flow and coronary vascular resistance (CVR) during repeated partial occlusions and reperfusions of the LAD in exercised and in control dogs.* Data are given as means ± s.e.m., and measured at the 20 min of the occlusion. Flow values were also determined immediately after the re-opening (1 min) of the LAD and at the end (20 min) of the reperfusion period. *P<0.05 vs. baseline. CBF = coronary blood flow (cm $s^{-1}$), CVR = coronary vascular resistance (Hgmm $cm^{-1}$ s). Occl. 1 and 2 = low-flow ischaemic periods; R = reperfusion.

### *3.2.5. Effects of repeated occlusion-reperfusion cycles on regional myocardial contractile function in paced and in exercised dogs*

Opposite to controls, in dogs, subjected to cardiac pacing or physical exercise 24 h previously, the repeated ischaemia-reperfusion cycles resulted in immediate, maximal reduction in segmental shortening even during the first episode of ischaemia (**Tables 3 and 6** summarize the changes in EDL, ESL and %SS), and there were no further reductions in %SS during the subsequent ischaemic insults (**Figures 5 and 7**). Furthermore, in these dogs reperfusion induced a somewhat more marked post-ischaemic hypercontractility than in the controls. This „compensatory" increase in regional contractility of the non-ischaemic (LCX) region was somewhat more enhanced than in the controls.

| | Base | O 1 | R 1 | O 2 | R 2 |
|---|---|---|---|---|---|
| **Control** | | | | | |
| EDL | 10.00±0.00 | 10.8±0.31* | 10.34±0.18 | 10.87±0.28* | 10.27±0.16 |
| ESL | 8.83±0.07 | 10.08±0.36* | 9.2±0.17 | 10.32±0.32* | 9.14±0.15 |
| %SS | 11.72±0.95 | 6.91±1.15* | 11.04±1.0 | 5.17±1.02*○ | 11.03±1.09 |
| **Exercised** | | | | | |
| EDL | 10.00±0.00 | 10.75±0.25* | 10.53±0.18 | 10.82±0.20* | 10.57±0.15 |
| ESL | 8.86±0.13 | 10.24±0.33* | 9.41±0.24 | 10.34±0.26* | 9.42±0.22 |
| %SS | 11.38±1.29 | 4.84±1.22*■ | 10.7±1.26 | 4.53±1.0* | 10.92±1.25 |

**Table 6.** *Changes in myocardial end-systolic (ESL) and end-diastolic lengths (EDL), and in segmental shortening (%SS)* within the ischaemic area during repeated partial occlusions and reperfusions of the LAD in exercised dogs. Data are given as means ± s.e.m., measured at the end of each occlusion (20 min) and reperfusion cycle. *P < 0.05 vs. baseline; ■P <0.05 vs. control group, ○P<0.05 vs. O 1. O = low-flow ischaemia; R = reperfusion.

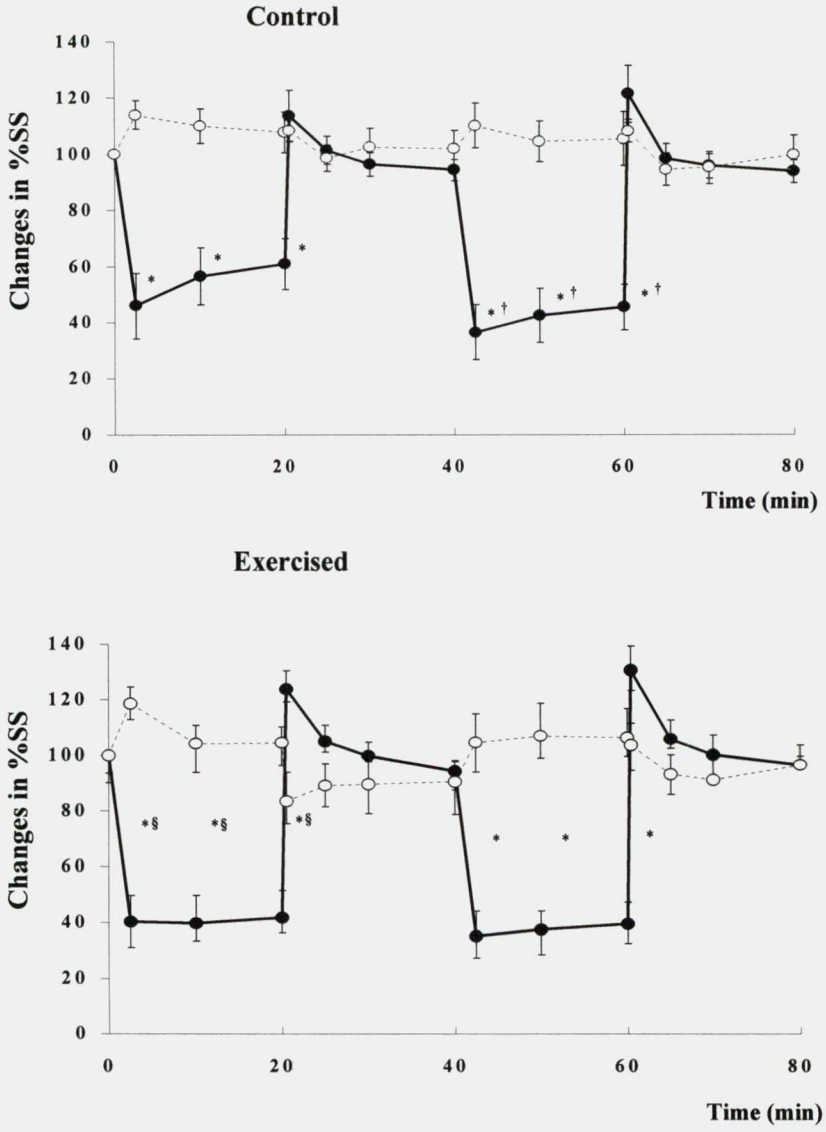


**Figure 7.** *Relative changes (in percent of the baseline values) in segmental shortening* within the ischaemic area (supplied by the LAD; filled circles) and non-ischaemic area (supplied by the LCX; open circles) during two consecutive low-flow ischaemia and reperfusion insults in exercised and control dogs. Values are means ± s.e.m. $^{*}P<0.05$ vs. baseline; $^{\dagger}P<0.05$ vs. 20 min; $^{\S}P<0.05$ vs. Control.

*3.2.6. Changes in myocardial tissue blood flow during repeated occlusions and reperfusions*

The results are summarised in **Table 7**. There were no significant differences between control and paced dogs as regard the reductions of tissue blood flow during repeated ischaemic episodes. The relationship between local myocardial blood perfusion and contractility under basal conditions and during repeated ischaemic interventions in the two groups is illustrated in **Figure 8**.

| | Baseline | Occlusion 1 | Occlusion 3 | Occlusion 4 |
|---|---|---|---|---|
| **Control group** | | | | |
| *Ischaemic area* | | | | |
| endocardial | 124.6±10.8 | 72.9±5.1 | 69.9±5.4 | 67.6±6.0 |
| epicardial | 123.4±11.9 | 87.6±7.4 | 85.3±5.6 | 85.9±6.1 |
| endo/epi ratio | 1.02±0.04 | 0.84±0.05 | 0.82±0.04 | 0.78±0.05 |
| *Non-ischaemic area* | | | | |
| endocardial | 122.5±10.8 | 123.5±10.4 | 118.5±9.4 | 116.8±10.5 |
| epicardial | 118.7±14.0 | 114.9±13.9 | 110.9±13.2 | 111.3±13.4 |
| endo/epi ratio | 1.06±0.06 | 1.11±0.07 | 1.11±0.08 | 1.09±0.08 |
| **Paced group** | | | | |
| *Ischaemic area* | | | | |
| endocardial | 104.6±5.7 | 60.0±9.1 | 57.9±8.5 | 53.5±8.0 |
| epicardial | 110.7±5.6 | 72.6±7.0 | 72.5±6.6 | 68.2±6.1 |
| endo/epi ratio | 0.94±0.01 | 0.80±0.08 | 0.78±0.07 | 0.76±0.08 |
| *Non-ischaemic area* | | | | |
| endocardial | 97.0±5.8 | 99.2±4.8 | 98.1±7.6 | 94.1±9.4 |
| epicardial | 106.8±7.9 | 105.0±6.2 | 109.7±6.5 | 106.8±5.7 |
| endo/epi ratio | 0.91±0.02 | 0.95±0.02 | 0.90±0.03 | 0.89±0.05 |

**Table 7.** The effects of successive partial occlusions of the left anterior descending coronary artery on myocardial tissue blood flow within the ischaemic and non-ischaemic area in control and in paced dogs (n = 4 for each group). Data are expressed in ml $min^{-1}$ 100 $g^{-1}$. Values are means ± s.e.m.

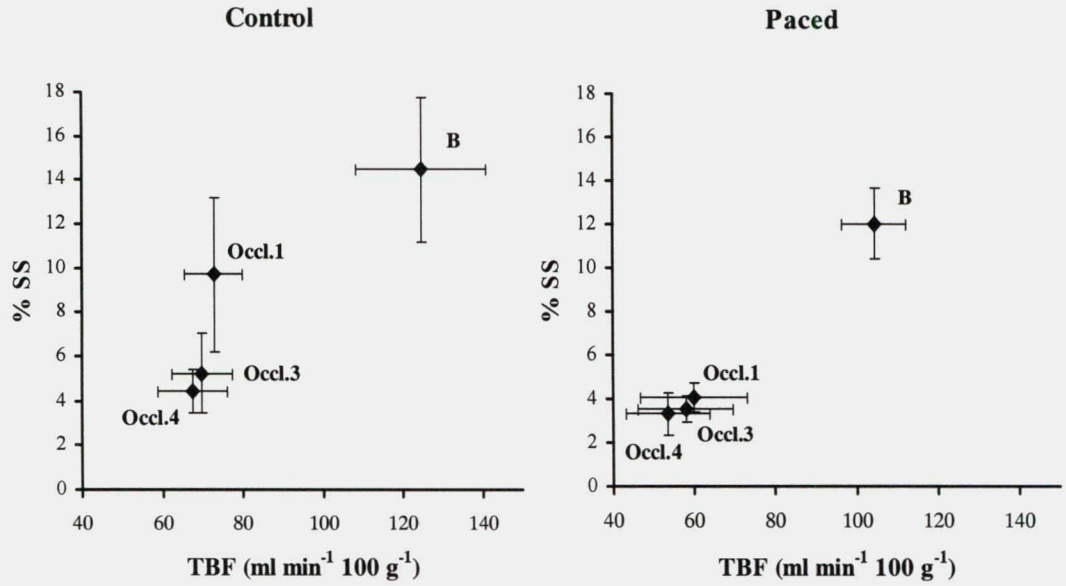


**Figure 8.** Relationship between tissue blood flow and segmental contractility within the ischaemic area measured in four control and in four paced dogs. Pacing abolished the shift in perfusion-contraction matching seen in the control group during the first (O 1) and the last two episodes (O 3 and 4) of low-flow ischaemia. Values are means ± s.e.m. Abbreviations: %SS = segmental shortening (%); TBF = tissue blood flow (ml min-1 100 $g^{-1}$); B = baseline; O = partial occlusion.

*3.2.7. Histological examinations*

There were no detectable histological lesions in myocardial samples taken from control and paced dogs. In the ischaemic tissue samples there were focal depletions in glycogen-content of the myofibrils within the sharply delineated subendocardial region. Such ultrastuctural changes were not seen in the non-ischaemic areas. There were no marked differences in histology between the control and paced dogs.

## 3.3. The role of nitric oxide (NO) in functional changes induced by repeated low-flow ischaemic episodes

L-NAME, an inhibitor of nitric oxide synthases (eNOS and iNOS), was given in intracoronary infusion in control and in paced dogs, in order to evaluate the contribution of NO in contractility changes during ischaemia / reperfusion cycles. **Figure 9.** shows that the 10 $\mu g\ kg^{-1}\ min^{-1}$ dose of L-NAME markedly reduced the vasodilator response of bradykinin, indicating an effective inhibition of bradykinin-induced generation of NO by NOS. This was similar in control and in paced dogs.

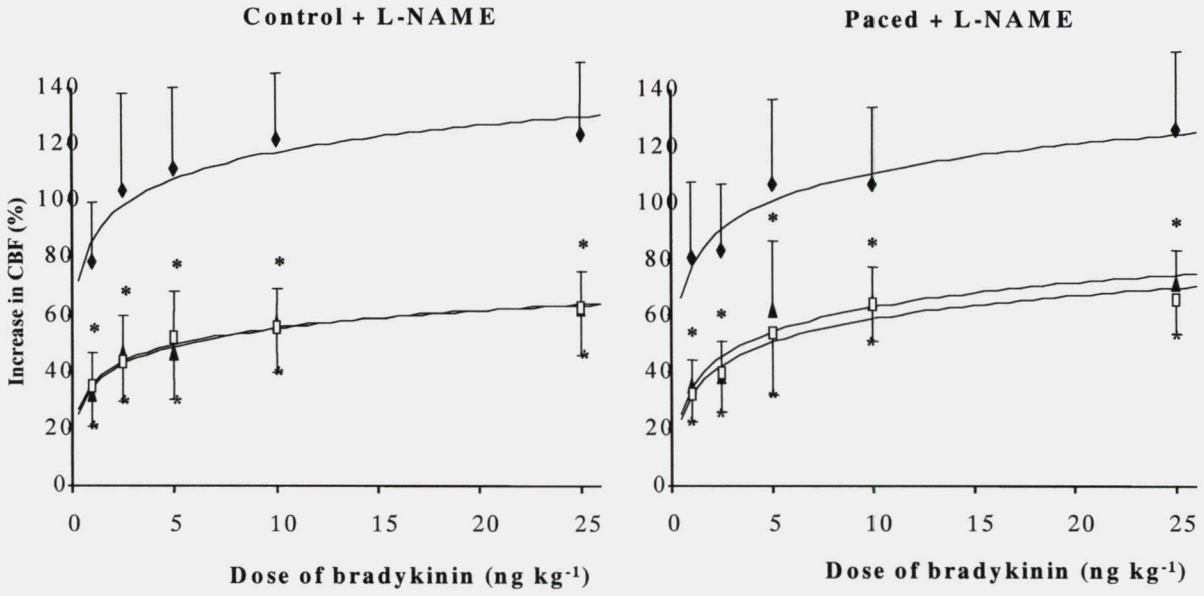


**Figure 9.** *Changes in coronary blood flow following increasing doses of bradykinin* (1, 2.5, 5, 10, 25 ng $kg^{-1}$) before (filled lozenges), and 1 min (open squares) and 40 min (filled triangles) after administration of L-NAME. Values are means ± s.e.m.

*3.3.1. Effects of L-NAME on the haemodynamic, coronary blood flow and vascular resistance and on the local contractile parameters*

Although systemic haemodynamic parameters were not significantly influenced by the intracoronary infusion of L-NAME, coronary vascular resistance increased in the controls (from 2.64 ± 0.24 to 3.12 ± 0.31 mmHg $cm^{-1}s$) and in the paced dogs (from 2.00 ± 0.27 to 2.30 ± 0.31 mmHg $cm^{-1}s$; $P<0.05$). These increases in resistance were returned to pre-infusion levels prior to the commencement of the first coronary artery occlusion (**Table 2**). Systolic segmental shortening decreased in both groups following administration of L-NAME (from 13.5 ± 1.3% to 12.9 ± 1.2 % in the control group and from 14.2 ± 1.1% to 13.7 ± 1.1 % in the paced group ($P<0.05$).

*3.3.2. Haemodynamic changes during repeated partial occlusions of the LAD in L-NAME-treated dogs*

There were no significant differences between paced and control dogs in the haemodynamic parameters during repeated ischaemia / reperfusion cycles (**Table 1**).

*3.3.3. Changes in coronary blood flow and coronary vascular resistance following repeated ischaemia-reperfusion cycles; effects of L-NAME*

These results are given in **Table 2**. In these L-NAME-treated groups, repeated constrictions of the LAD resulted in an approximately 50 % reductions in blood flow during each ischaemic period. There was however an interesting observation in the L-NAME-treated dogs during reperfusion; i.e. the post-occlusion hyperaemia in both the paced (about 3.2 fold increase in CBF vs. 4 fold in paced without L-NAME; $P<0.05$) and the control group (about 2.2 fold increase in CBF vs. 3 fold in controls without L-NAME; $P<0.05$) was less marked than in the untreated control and paced dogs. This hyperaemic reaction was almost the same during the consecutive reperfusions.

Coronary vascular resistance (CVR) measured at baselinein the L-NAME-treated dogs were not significantly different from the not treated groups (**Table 2**). Increase in CVR during the first partial occlusion was more pronounced in control dogs given L-NAME ($P<0.05$ vs. controls), although not significantly different in the paced + L-NAME group compared to

those dogs that were paced without L-NAME treatment. During reperfusion CVR returned near to the baseline values. Changes induced by partial occlusions two to four, and also by reperfusion, were similar to those that occurred during and after the first low-flow ischaemic episode.

*3.3.4. The assessment of the involvement of nitric oxide in changes of local myocardial contractile function during repeated low-flow ischaemia / reperfusion cycles*

The local administration of L-NAME prior to the repeated occlusion-reperfusion cycles markedly attenuated the changes in %SS both in paced and control dogs (**Table 3, Figure 6**). Pretreatment with L-NAME abolished the adaptive effects of cardiac pacing on segmental contractility. Similarly, in the presence of L-NAME, there were less marked and similar reductions in %SS within the ischaemic segment in the control dogs during the consecutive partial occlusions. Furthermore, the 'compensatory increase' in contractility, that was apparent within the normal, non-ischaemic segment during repeated occlusions especially in the paced dogs, was much reduced following the administration of L-NAME, as was the hypercontractility that resulted from reperfusion of the ischaemic area (**Figure 5.**). Further, the mild postischaemic myocardial depression (stunning) which could be observed at the end of the reperfusion cycles, and which was maintained until the next ischaemic insult, was blunted in dogs treated with L-NAME.

*3.3.5. Changes in lactate production during repetitive ischaemia / reperfusion cycles in control dogs, in paced dogs and after L-NAME administration*

Under normal conditions there were no significant differences between the groups in lactate extraction ratios (LER; 13 ± 4 % in control dogs, 14 ± 7 % in paced dogs, 21 ± 2% in paced dogs given L-NAME and 17 ± 1 % in control dogs given L-NAME). The first episode of ischaemia resulted in similar changes in LER (lactate production) in both control and paced dogs (-33 ± 26 % and -32 ± 7 %; respectively). In contrast, during the subsequent three ischaemic cycles, LER was decreased only in the controls (-21 ± 15 %, -20 ± 12 % and -12 ± 14 %; respectively); in the paced dogs it remained unchanged (-32 ± 7 %, -30 ± 13 % and -28 ± 9 %, respectively). Following administration of L-NAME, lactate production was markedly increased in both groups. Thus, in the paced dogs, LERs were -67 ± 18 %, -61 ± 13 %, -69 ±

25 % and -68 ± 18 %, respectively and in the controls these were -84 ± 4 %, -81 ± 10 %, -65 ± 13 % and -67 ± 10 % during partial occlusions 1 to 4, respectively.

### *3.3.6. Assessment of the area at risk and the severity of ischaemia during repeated partial occlusions of the LAD*

The severity of myocardial ischaemia was assessed by changes in the ST-segment as recorded from the endocardial electrode within the ischaemic area. Repeated ischaemic interventions resulted in slight (2 to 4 mV) increases, which was similar in magnitude during the repeated partial occlusions in all groups.

There were no significant differences in the area at risk between the groups. Thus, the risk (ischaemic) area was 32.9 ± 1.1 % in the controls, 31.4 ± 1.0 % in the paced, 34.2 ± 1.4 % in the exercised group, and 31.3 ± 1.0 % in the sham-operated but not exercised control dogs. Similarly, the risk area was 35.4 ± 1.1 % in the paced + L-NAME and 33.1 ± 1.5 % in the control + L-NAME groups.

# 4. DISCUSSION

## 4.1. New findings

I. We described in anaesthetized open chest dogs that repeated partial coronary occlusions result in progressively decreasing regional contractile function within the ischaemic area (until a definite grade), without any significant changes in the resting segmental contractility during reperfusion.

II. We showed for the first time that 24 h after cardiac pacing, decrease in segmental shortening is more enhanced already during the first episode of low-flow ischaemia / reperfusion cycles and no further decrease occur.

III. These adaptive changes in regional contractile function were abolished both in the early and late phases of preconditioning after the local administration of L-NAME, a non-selective inhibitor of NO synthesis, resulting in metabolic deterioration during ischaemia. This might indicate that the protective role of NO is an adaptive process.

IV. Finally, we demonstrated that, as with cardiac pacing, a single episode of physical exercise, 24 hours prior to ischaemia, results in immediate maximal reduction in segmental contractility that occur during the first ischaemic episode. This may represent a form of cardiac preconditioning of clinical importance, indicating that the myocardium rapidly adapts to flow changes by reducing its contractility and thereby its energy and oxygen (metabolic) demands.

## 4.2. Effects of preconditioning on segmental contractile function during repeated episodes of low-flow ischaemia

Our studies demonstrate that in anaesthetized dogs, repeated low-flow ischaemic episodes induce a progressive reduction in systolic contractile function of the ischaemic myocardial segment. The maximum reduction occurs during the third episode of ischaemia, and an additional (fourth) ischaemic insult results in no further decrease in contractility. Regional contractility always returned near to the basal value following reperfusion, indicating that no myocardial stunning developed in this model following the repeated

ischaemic insults. We may conclude, that the myocardium is able to adapt to the repeated ischaemic stress by reducing its contractile function and thereby oxygen and metabolic demands. This is also reflected in reduced lactate production during ischaemia. This functional adaptation to ischaemic stress would enhance the possibility of cell viability during a subsequent ischaemic episode. In other words, the early ischaemic insults may serve as preconditioning stimuli for the heart in order to protect itself against the harmful consequences of the subsequent ischaemic events.

A similar adaptation was achieved when hearts had been subjected to cardiac pacing, 24 hours prior to the ischaemic cycles. This may represent a form of „delayed" preconditioning [45, 57]. Under these conditions the maximum observed reduction in ischaemic segmental contractile function was already apparent during the first low-flow ischaemic episode, which was much more enhanced than in the non-preconditioned hearts, and no further reductions occurred during the subsequent ischaemic insults (**Figure 5**). Previously, cardiac pacing was already demonstrated to provide effective early and delayed protection against ischaemic and reperfusion arrhythmias [45, 54, 57] and ischaemic myocardial necrosis [58, 94]. In our model we used this method to induce delayed preconditioning, because it – opposite to repeated short complete coronary occlusions – did not result in significant myocardial stunning, as it was apparent in the control and paced groups.

Two further findings are also of interest. First, the postocclusion hyperaemic response was more enhanced in the previously paced group, and it was accompanied by a larger transient hypercontractility of the reperfused area within the first few minutes of reopening the coronary artery (**Table 2, Figure 5**). Both changes were markedly attenuated following the inhibition of NO production due to L-NAME administration. Second, the „compensatory" increase in contractility, measured within the non-ischaemic (LCX) segment during ischaemia, was somewhat more marked in the paced dogs than in the controls (**Figure 5**). This increase in the contractile function of the non-ischaemic area is supported by the increase in CBF that normally occurs in the LCX branch when the anterior descending coronary artery is occluded ('compenastory vasodilation', [69]). Presumably, this increased segmental contractility within the normal myocardial region is responsible for the observation that only slight but staistically not significant changes occurred in the global myocardial contractile function (LV $dP/dt_{max}$) in each of the groups (**Table 1**), despite that the affected, ischaemic area of the left ventricular wall was substantial (more than 30% of the region supplied by the

LAD coronary artery). In both groups this compensation was abolished following administration of L-NAME.

We propose that the reduction in segmental contractility following repeated episodes of ischaemia (and 24 h after cardiac pacing) is due to a more economical metabolism rather than to any cumulative mechanical or ultrastructural damages that might have been expected following ischaemia. Certainly, there was no evidence of structural alterations in the contractile apparatus in our histological studies. Furthermore, no significant changes occurred in the collateral blood flow during repeated occlusions. Myocardial tissue blood flow, measured by the 'coloured microspheres' method, was similar at any level of contractile dysfunction in both groups. Indeed, the only indication that any coronary collaterals had opened, occurred in control dogs, when segmental contractility during the first partial occlusion was improved by the end of the ischaemic period (**Figure 5**). This was not observed either during subsequent occlusions in the controls or in the paced dogs. If collateral recruitment had played a role in this adaptive phenomenon then an improvement in contractility, rather than a depression would have been expected during consecutive ischaemic events. This is consistent with previous studies, showing no significant changes in collateral blood flow during repeated periods of ischaemia in animals [88] and in man [89]. Similarly, there were no significant changes in the endocardial/epicardial flow ratio during repeated partial occlusions or 24 h after cardiac pacing. Thus, the adaptation in contractile function is not due to an altered endocardial blood supply, which markedly influences the contractile force of the ischaemic segment. Our results support the previous findings of others [50].

### 4.3. The role of NO in the contractile adaptation during ischaemia

Végh and colleagues have shown previously that nitric oxide plays an important role in dogs against ischaemic and reperfusion ventricular arrhythmias both in the early [97] and the delayed [98-100] phases of preconditioning. We have also proposed an increased formation of NO during ischaemia following preconditioning, which may contribute to the protection. The evidence for this comes from the experiments in which L-NAME was administered to both control and paced dogs prior to the repeated ischaemic challenges; this abolished the marked reductions in segmental contractility in both groups. Furthermore, L-NAME attenuated the hyperaemic reactions and transient hypercontractility that occurred

within the ischaemic region immediately after opening of the obstruction. Thus, we suppose that the early ischaemic insults as well as cardiac pacing, 24 hours previously (i.e. the delayed phase of preconditioning), may enhance NO production, which results in progressive or more enhanced reductions in contractility during the repeated ischaemic insults and contributes to the marked increase in blood flow following reperfusion (post-occlusion hyperaemia), also suggesting NO involvement in the coronary vascular responses following ischaemia. Furthermore, the marked elevation in lactate production following L-NAME administration suggests that inhibition of NO generation not only abolishes the adaptive reduction in contractility but also increases anaerobic metabolism in the ischaemic tissue. Similarly, in more studies NO was found to decrease the ischaemic challenge of the myocardium [101-103] and oxygen consumption [104], resulting in better metabolic conditions and decreased lactate production during ischaemia.

### *4.3.1. Possible mechanisms by which NO contributes to the adaptive changes in contractility*

Ischaemia elevates the intracellular amount of NO through activating the $Ca^{2+}$-sensitive nitric oxide synthase enzyme (cNOS; [105-110]. Furthermore, upregulated cNOS activity [111] and enhanced production of bradykinin during ischaemia was observed after preconditioning [112], which may further enhance NO release during a subsequent ischaemia [108]. Newly synthetized enzymes also contribute to the more pronounced NO production; elevation in the amount of the cNOS protein was described 30 min after a hypoxic challenge [110], presumably through the hypoxic regulation of mRNA transcription. Nuclear factor-kappa-B (NFκB) appears in the myocardium after preconditioning [113, 114], which may refer to *de novo* synthesis of the inducible form of NOS (iNOS) in the cardiomyocyte [115, 116], contributing to the enhanced NO production during ischaemia in the preconditioned myocardial tissue.

Although the precise mechanism responsible for the reduced myocardial function during low-flow ischaemia remains unclear, a significant role for adenosine [50] and nitric oxide [106] has been proposed. Thus, in patients with effort angina, endogenous NO reduced myocardial contractile function and improved metabolic function of the heart [106]. The results of Hori's group, who used complete coronary artery occlusion, confirmed that the increased generation of NO during ischaemia may contribute to the reduced contractile response [50, 105, 106, 108]. Furthermore, Heusch and colleagues found in anaesthetized pigs

that endogenous NO contributes to hibernation by reducing myocardial oxygen consumption [117]. This would help to maintain regional myocardial contractile function during ischaemia.

The effects of NO on myocardial contractility are still under debate. There is evidence for both the negative or the positive inotropic effects of nitric oxide which is most probable concentration dependent [for a detailed review see 116]. It seems, that in smaller intracellular concentrations (<1 μM) NO enhances, whereas in larger amount decreases contractility. The elevated amount of endogenous NO during ischaemia (e.g. following preconditioning) [105, 107, 108, 117, 119] initiates the intracellular production of cyclic GMP through activation of the soluble guanylate cyclase [120, 121], which in turn activates the protein kinase G [114], resulting in the desensitization of the myofilaments to calcium [122]. Further, cGMP was found to decrease calcium influx through the L-type calcium channels [123]. In addition, NO was observed to directly inhibit the L-type calcium channels. Recently, an isoform of neuronal NOS was described to be located on the sarcoplasmic reticulum, which also might modulate $Ca^{2+}$ ion transport of the sarcoplasmic reticulum [124]. Méry et al. described an additional action of cGMP, which in higher concentrations activates the phosphodiesterase 2 ($PDE_2$), leading to a decrease in the concentration of cAMP and modifieing L-type calcium-current, which in turn further decreases the contractile response [125].

Nitric oxide, derived in part from endothelial cells [126, and reviewed in 127], influences myocardial metabolism during ischaemia by reducing myocardial oxygen consumption [126, 128, 129]. The most important target seems to be cytochrom c oxidase [109, 130, 131] which is blocked reversibly already at small NO-concentrations (10-100 nM), in particular if the intracellular oxygen suppply is largely decreased [132]. Other enzymes in mitochondrial respiration were also found to be inhibited after an exposure to NO, e.g. complex I, complex II and aconitase [126]. Others confirmed a decrease in oxygen consumption through the inhibition of the electron transport chain following NO exposure [101]. The effect of NO in reducing oxygen consumption seems to be a direct effect, independent of cGMP [126]. Recently, a mitochondrial $Ca^{2+}$-sensitive isoform of NOS was described [133], which may also contribute to the regulation of oxygen consumption. The blockade of myocardial respiration leads to reduced production of ATP during ischaemia, which might be, in part, responsible for the contractile dysfunction [134]. Although neither oxygen consumption, nor glucose uptake were measured in our present sudy, lactate extraction data show that inhibition of NO formation increased anaerobic metabolism.

In our studies, L-NAME administriation after cardiac pacing did not completely abolish the enhanced reduction in contractility during low-flow ischaemic episodes if it is compared to the not paced dogs. Presumably, NO is not the only mediator in the late phase of preconditioning which might mediate contractile adaptation during ischaemia. After preconditioning a significant increase in adenosine-release during low-flow ischaemia was described, which also contributes to the negativ inotropic effects [50, 135]. Although, we did not examine the role of adenosine in our experiments, the involvement of other mechanisms, or mediators, than NO, in the adaptive changes can not be ruled out.

## 4.4. Effects of physical exercise, as a preconditioning stimulus on myocardial contractility during ischaemia

There is a good deal of experimental and clinical evidence that physical exercise, as a „beneficial" stress is cardioprotective [68, 69, 136-139, for a detailed review see 140]. However, there is less known about the potential mechanisms of this exercise-induced cardioprotective effect. The available data suggest, that there are no susbstantial differences between the basic mechanisms of the early and late phases of protection induced by various preconditioning stimuli (i.e. short coronary occlusions, cardiac pacing or physical exercise). There are, of course, important differences between the effects, by which the heart rate is elevated during cardiac pacing or physical exercise, there is, however, common that both following cardiac pacing and exercise, the cardiac pressure load and the oxygen demand of the myocardium increases and an imbalance in oxygen supply-demand occures, which results from transient hypoxia within the myocardium, mostly in the endocardial layer.

It is proposed, that during exercise, several endogenous mediators are released (e.g. NO, chatecholamines, opioid peptides, prostanoids, bradykinin, reactive oxygen species) which almost certainly contribute to the cardioprotective effects. There is a number of evidence that NO is released during physical activity both in animals [104, 141], and in man [119, 142] and induces preconditioning-like protection. For example, we have shown that a single episode of physical exercise resulted in a 3 fold increase in iNOS activity 24 h later [69], when a marked antiarrhythmic effect had been detected during ischaemia and reperfusion. This effect was sensitive to aminoguanidin, a selective inhibitor of iNOS.

Already more than two hundred years ago, a relationship between the severity of anginal pains and the physical activity of patients was observed [143]. Similarly, physical exercise has been found to decrease the incidence of sudden cardiac death, and cumulative cardiac death [144, 145], as well as to reduce the extent of ischaemic myocardial necrosis and to attenuate the complications of ischaemic heart disease [146]. Clinical studies revealed that physical exercise test in patients resulted in a better tolerance to ischaemia if a primary exercise was performed (24 or 48 h previously); i.e. more time was required to reach the same level of ischaemic changes (ECG signs and / or anginal pain), whereas the myocardial oxygen consumption significantly decreased [139, 147-149]. In other studies, in patients, who underwent physical exercise test 24 h before angioplasty, there were less severe changes in ST segment during subsequent PTCA [138, 150] and this adaptation was independent of the collateral network [138].

However, there are few, and conflicting data about the effects of repeated physical stress or other ischaemic challenges on the contractile function in patients with ischaemic heart disease. Some studies presume – mostly based on theoretical explanations – that myocardial contractile function might decrease during ischaemic interventions following preconditioning and this results in a better metabolic condition for the heart [151-153]. In contrast, other studies found an improved segmental contractile function within the ischaemic region following preconditioning or repetitive ischaemia [154, 155]. It is clear that further experimental and clinical studies are required to give correct answer for these questions.

## 4.5. Conclusions

Repeated partial occlusions of a main coronary artery result in a progressive reduction in contractility whithin the ischaemic myocardium. This decreased contractility might provide a better cell viability during the subsequent ischaemic episodes. In other words, the early ischaemic insults may serve as preconditioning stimuli. Similarly, cardiac pacing or physical exercise 24 h prior to such ischaemic challenges evoke this adaptive contractile response. We suggest that the reduction in segmental contractility protects the myocytes by reducing the proportion of oxygen expended in contractility, thus promoting myocyte viability. It is therefore a protective adaptation designed to reduce myocyte injury during ischaemia. Our results also suggest that this adaptation might be mediated by nitric oxide.

A question could be raised whether this adaptation (decrease in regional contractile function within the ischaemic area) is beneficial for the heart or for the whole organism. In our experimental model the area of the left ventricular wall that affected by ischaemia was substantial (the region supplied by the LAD was more than 30%), however this did not materially influenced global left ventricular function (LV $dP/dt_{max}$) and haemodynamic status. This suggests the importance of the compensatory changes that occur in the normal (non-ischaemic) myocardial region of the left ventricle. We believe that a decrease in regional contractility during ischaemia following preconditioning is protective for the myocardium – at least if the ischaemia does not involve a very large amount of myocardium. Although we do not know whether the same adaptive process would perform, if substantially more myocytes or a previously damaged heart were affected by ischaemia.

If we are correct in our hypothesis that preconditioning enables myocardium to adapt in such a way that cellular viability is maintained during ischaemia, the question arises as to whether this has a clinical significance. We think, it does. Successive reductions in coronary blood flow commonly occur in patients with coronary artery disease and these do not necessarily result from complete brief coronary artery occlusions; as we have shown, partial occlusions also result in significant downstream reductions in flow. These would result in reductions in contractility in the area supplied by the partially occluded vessel. The myocardium responds in two ways. First, the initial ischaemia acts as a preconditioning stimulus to reduce the effects of subsequent ischaemic periods. Second, a stimulus, that results in delayed preconditioning (cardiac pacing or exercise), has aleady prepared the heart to withstand later brief ischaemic periods by immediately reducing contractility (and thus sparing oxygen consumption) in the affected segment, thus shifting oxygen availability towards maintaining cellular integrity and survival.

Of clinical importance, physical exercise may provide a form of preconditioning which is easy to perform. Further regular physical exercise would maintain the myocardium in permanently protected status.

## 5. REFERENCES


1. Hearse DJ. Myocardial ischaemia: Can we agree on a definition for the 21st century? *Cardiovasc Res 1994; 28:1737-1744*
2. Jennings RB, Ganote CE, Reimer KA. Ischaemic tissue injury. *Am J Pathol 1975; 81:179-198*
3. Reimer KA, Jennings RB, Hill ML. Total iscemia in dog hearts in vivo 2. High energy phosphate depletion and associated defects in energy metabolism, cell volume regulation, and sarcolemmal integrity. *Circ Res 1981; 49: 901-911*
4. Jennings RB, Reimer KA, Steenbergen Jr. C, Schaper J. Total ischaemia III: Effect of inhibition of anaerobic glycolysis. *J Mol Cell Cardiol 1989; 21 (Suppl 1): 37-54*
5. Tenant R, Wiggers CJ. The effects of coronary occlusion on myocardial contraction. *Am J Physiol 1935; 112:351-361*
6. Heusch G, Schulz R. Myocardial function during and after myocardial ischaemia. *J Cardiovasc Pharmacol 1996; 28(Suppl. 2):S9-S24*
7. Reimer KA, Jennings RB. The "wavefront phenomenon" of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab Invest 1979; 40:633*
8. Opie LH. The multifarious spectrum of ischaemic left ventricular dysfunction: Relevance of new ischaemic syndromes. *J Mol Cell Cardiol 1996; 28:2403-2414*
9. Yellon DM, Rahimtoola SH, Opie LH. New Ischemic Syndromes. *Authors' Publishing House, New York; 1997*
10. Braunwald E, Kloner RA. The stunned myocardium: prolonged, postischemic ventricular dysfunction. *Circulation 1982; 66:1146-1149*
11. Kloner RA, Bolli R, Marban E, Reinlib L, Braunwald E, Participants. Medical and cellular implications of stunning, hibernation, and preconditioning. An NHLBI workshop. *Circulation 1998; 97:1848-1867*
12. Ferrari R, Ceconi C, Curello S, Percoco G, Toselli T, Antonioli GE. Ischaemic preconditioning, myocardial stunning, and hibernation: Basic aspects. *Am Heart J 1999; 138:S61-S68*
13. Rahimtoola SH. A perspective on the three large multicenter randomized clinical trials of coronary bypass surgery for chronic stable angina. *Circulation 1985; 72:V-123-V-135*
14. Rahimtoola SH. The hibernating myocardium. *Am Heart J 1989; 117:211-221*
15. Heusch G, Ferrari R, Hearse DJ, et al. "Myocardial hibernation": questions and controversies. *Cardiovasc Res 1997; 36:298-300*
16. Ferrari R, Ceconi C, Curello S, Benigno M, La Canna G, Visioli O. Left ventricular dysfunction due to the new ischaemic outcomes: Stunning and hibernation. *J Cardiovasc Pharmacol 1996; 28(Suppl. 1):S18-S26*
17. Pantely GA, Malone SA, Rhen WS, Anselone CG, Arai A, Bristow J, Bristow JD. Regeneration of myocardial phosphocreatine in pigs despite continued moderate ischemia. *Circ Res 1990; 67:1481-1493*
18. Depre C, Vanoverschelde JLJ, Melin JA, Borgers M, Bol A, Ausma J, Dion R, Wijns W. Structural and metabolic correlates of the reversibility of chronic left ventricular ischemic dysfunction in humans. *Am J Physiol 1995; 268:H1265-H1275*
19. Elsasser A, Schaper J. Hibernating myocardium: adaptation or degeneration?. *Basic Res Cardiol 1995; 90:47-49*
20. Borges M, Ausma J. Structural aspects of the chronic hibernation myocardium. *Am J Cardiol 1995; 90:44-46*
21. Elsasser A, Schlepper M, Klövekorn WP, Cai W, Zimmermann R, Müller KD, Strasser R, Kostin S, Gagel C, Münkel B, Schaper W, Schaper J. Hibernating myocardium. An incomplete adaptation to ischemia. *Circulation 1997; 96:2920-2931*
22. Ross Jr. J. Myocardial perfusion-contraction matching. Implications for coronary heart disease and hibernation. *Circulation 1991; 83:1076-1083*

23. Ferrari R, Ferrari F, Benigni M, Pepi P, Visioli O. Hibernating myocardium: Its pathophysiology and clinical role. *Mol Cell Biochem 1998; 186:195-199*
24. Heusch G. Hibernating myocardium. *Physiol Res 1998; 78:1055-1085*
25. Liu Y, Sato B, O'Rourke B, Marban E. Mitochondrial ATP-dependent potassium channels. Novel effectors of cardioprotection?. *Circulation 1998; 97:2463-2469*
26. Berry GJ, Masek M. The pathology of hibernating myocardium. *Nucl Med Commun 2002; 23:303-309*
27. Kolar F, Ostadal B. Molecular mechanisms of cardiac protection by adaptation to chronic hypoxia. *Physiol Res. 2004; 53 Suppl 1:S3-13*
28. Canty JM. Nitric oxide and short-term hibernation. Friend or foe? *Circ Res 2000; 87:85-87*
29. Heusch G, Schulz R, Rahimtoola SH. Myocardial hibernation: a delicate balance. *Am J Physiol. 2005; 288:H984-99*
30. Billo R, Zughaib M, Li X-Y, Tang X-L, Sun J-Z, Triana JF, McCay P. Recurrent ischaemia in the canine heart causes recurrent bursts of free radical production that have a cumulative effect on contractile function: a pathophysiological basis for chronic myocardial 'stunnung'. *J Clin Invest 1995; 96:1066-1084*
31. Homans DC, Laxon DD, Sublett E, Lindstrom P, Bache RJ. Cumulative deterioration of myocardial function after repeated episodes of exercise-induced ischemia. *Am J Physiol 1989; 256:H1462-H1471*
32. Sun JZ, Tang XL, Knowlton AA, Park SW, Qiu Y, Bolli R. Late preconditioning against myocardial stunning. An endogenous protective mechanism that confers resistance to postischemic dysfunction 24 h later after brief ischemia in conscious pigs. *J Clin Invest 1995; 95:388-403*
33. Shen YT, Vatner SF. Mechanism of impaired myocardial function during progressive coronary stenosis in conscious pigs. Hibernation versus stunning? *Circ Res 1995; 76:479-488*
34. Bolli R. Myocardial 'stunning' in man. *Circulation 1992; 1671-1691*
35. Di Carli MF, Prcevski P, Singh TP, Janisse J, Ager J, Muzik O, Vander Heide R. Myocardial blood flow, function, and metabolism in repetitive stunning. *J Nucl Med 2000; 41:1227-1234*
36. Canty JM, Fallavollita JA. Hibernating myocardium represents a primary downregulation of regional myocardial oxygen consumption distal to a critical stenosis. *Basic Res Cardiol 1995; 90:5-8*
37. Bonow RO. Identification of viable myocardium. *Circulation 1996; 94:2674-2680*
38. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation 1986; 74:1124-1136*
39. Schott RJ, Rohmann S, Braun ER, Schaper W. Ischemic preconditioning reduces infarct size in swine myocardium. *Circ Res 1990; 66:1133-1142*
40. Liu Y, Downey JM. Ischemic preconditioning protects against infarction in rat heart. *Am J Physiol 1992; 263:H1107-H1112*
41. Komori S, Parratt JR, Szekeres L, Vegh A. Preconditioning reduces the severity of ischaemia and reperfusion-induced arrhythmias in both anaesthetized rats and dogs. *J Physiol 1990; 423: 16P*
42. Végh Á, Szekeres L, Parratt JR. Protective effects of preconditioning of the ischaemic myocardium involve cyclooxigenase products. *Cardiovasc Res 1990; 24:1020-1023*
43. Vegh A, Szekeres L, Parratt JR. Preconditioning of the ischaemic myocardium; involvement of the L-arginine nitric oxide pathway. *Br J Pharmacol 1992; 107:648-652*
44. Shiki K, Hearse DJ. Preconditioning of ischaemic myocardium: reperfusion-induced arrhythmias. *Am J Physiol 1987; H1470-H1476*
45. Kaszala K, Vegh A, Papp JG, Parratt JR. Time-course of the protection against ischaemia and reperfusion-induced ventricular arrhytmias resulting from brief periods of cardiac pacing. *J Mol Cell Cardiol 1996; 28:2085-2095*
46. Cave AC. Preconditioning induced protection against post-ischemic contractile dysfunction: characteristics and mechanisms. *J Mol Cell Cardiol 1995; 27:969-979*

47. Schaper W. Molecular mechanisms in "stunned" myocardium. *Cardiovasc Drugs Ther 1991; 5:925-932*
48. Urabe K, Miura T, Iwamoto T, Ogawa T, Goto M, Sakamoto J, Iimura O. Preconditioning enhances myocardial resistance to postischemic myocardial stunning via adenosine receptor activation. *Cardiovasc Res 1993; 27:657-662*
49. Kaplan LJ, Bellows CF, Blum H, Mitchell M, Whitman GJR. Ischaemic preconditioning preserves end-ischaemic ATP, enhancing functional recovery and coronary flow during reperfusion. *J Surg Res 1994; 57:179-184*
50. Minamino T, Kitakaze M, Sato H, Funaya H, Ueda Y, Asanuma H, Kuzuya T, Hori M. Effects of ischaemic preconditioning on contractile and metabolic function during hypoperfusion in dog. *Am J Physiol 1998; 274:H684-H693*
51. Tanoue Y, Herijgers P, Meuris B, Verbeken E, Leunens V, Lox M, Flameng W. Ischemic preconditioning reduces unloaded myocardial oxygen consumption in an in-vivo sheep model. *Cardiovasc Res. 2002; 55:633-41*
52. Richard B, Blanc T, Kaeffer N, Tron C, Thuillez C. Myocardial and coronary endothelial protective effects of acetylcholine after myocardial ischaemia and reperfusion in rats: role of nitric oxide. *Br J Pharmacol 1995; 115:1532-1538*
53. Yellon DM, Alkhulaifi AM, Pugsley WB. Preconditioning the human myocardium. *Lancet 1993; 342:276-277*
54. Vegh A, Szekeres L, Parratt JR. Transient ischemia induced by rapid cardiac pacing results in myocardial preconditioning. *Cardiovasc Res 1991; 25:1051-1053*
55. Vegh A, Komori S, Szekeres L, Parratt JR. Antiarrhythmic effects of preconditioning in anaesthetized dogs and rats. *Cardiovasc Res 1992; 26:487-495*
56. Szekeres L, Papp JG, Szilvassy Z, Udvary E, Vegh A. Moderate stress by cardiac pacing may induce both short term and long term cardioprotection. *Cardiovasc Res 1993; 27:593-596*
57. Parratt JR, Vegh A. Delayed protection against ventricular arrhytmias by cardiac pacing. *Heart 1997; 78:423-425*
58. Domenech RJ, Macho P, Velez D, Sanchez G, Liu X, Dhalla N. Tachycardia preconditions infarct size in dogs. Role of adenosine and protein kinase C. *Circulation 1998; 97:786-794*
59. Ovize M, Przyklenk K, Kloner RA. Partial coronary stenosis is sufficient and complete reperfusion is mandatory for preconditioning the canine heart. *Circ Res 1992; 71:1165-1173*
60. Shizukuda Y, Mallet RT, Lee SC, Downey HF. Hypoxic preconditioning of ischaemic canine myocardium. *Cardiovasc Res 1992; 26:534-542*
61. Koning MMG, Simonis LAJ, de Zeeuw S, Nieukoop S, Post S, Verdouw PD. Ischaemic preconditioning by partial occlusion without intermittent reperfusion. *Cardiovasc Res 1994; 28:1146-1151*
62. Koning MMG, Gho BCG, van Klaarwater E, Duncker DJ, Verdouw PD. Endocardial and epicardial infarct size after preconditioning by a partial coronary artery occlusion without intervening reperfusion. Importance of the degree and duration of flow reduction. *Cardiovasc Res 1995; 30:1017-1027*
63. Ovize M, Kloner RA, Hale SL, Przyklenk K. Coronary cyclic flow variations "precondition" ischemic myocardium. *Circulation 1992; 85:779-789*
64. Iwamoto T, Bai XJ, Downey HF. Preconditioning with supply-demand imbalance limits infarct size in dog heart. *Cardiovasc Res 1993; 2071-2076*
65. Ovize M, Kloner RA, Przyklenk K. Stretch preconditions canine myocardium. *Am J Physiol 1994; 266:H137-H146*
66. Post H, Heusch G. Ischemic preconditioning. Experimental facts and clinical perspective. *Minerva Cardioangiol. 2002; 50:569-605*
67. Gross GJ. Pharmacological preconditioning: Potential new treatment modalities for the ischemic myocardium. *Vascul Pharmacol. 2005; 42:199*

*68.* Vegh A, Babai L, Kovacs K, Papp JG, Parratt JR. Exercise 24 h prior to coronary artery occlusion reduces arrhytmia severity in anaesthetized dogs: role of nitric oxide. *J Physiol 2000; 525P:14P*

*69.* Babai L, Szigeti Zs, Parratt JR, Végh Á. Delayed cardioprotective effects of exercise in dogs are aminoguanidine sensitive; possible involvement of nitric oxide. *Clin Sci 2002; 102:435-445*

*70.* Szigeti Zs, Simon K, Papp Gy, Végh Á. Effects of physical exercise on regional contractility during repeated low-flow ischaemic episodes in anaesthetized dogs. *Cardiologia Hungarica 2004; 34:241-248*

*71.* Yellon DM, Baxter GF, Garcia-Dorado D, Heusch G, Sumeray MS. Ischaemic preconditioning: present position and future directions. *Cardiovasc Res 1998; 37:21-33*

*72.* Kuzuya T, Hoshida S, Yamashita N, Fuji H, Oe H, Hori M, Kamada T, Tada M. Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. *Circ Res 1993; 72:1293-1299*

73. Kis A, Vegh A, Papp JG, Parratt JR. Repeated cardiac pacing extends the time during which canine hearts are protected against ischaemia-induced arrhythmias: role of nitric oxide. *J Mol Cell Cardiol 1999; 31:1229-1241*

*74.* Parratt JR. Endogenous myocardial protective (antiarrhythmic) substances. *Cardiovasc Res 1993; 27: 693-702*

*75.* Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev. 2003; 83:1113-51*

*76.* Sanada S, Kitakaze M. Ischemic preconditioning: emerging evidence, controvery, and translational trials. *Int J Cardiol 2004; 97: 263-76*

*77.* Ghosh S, Standen NB, Galinanes M. Evidence for mitochondrial KATP channels as effectors of human myocardial preconditioning. *Cardiovasc Res 2000; 45:934-940*

*78.* Hanley PJ, Daut J. K(ATP) channels and preconditioning: A re-examination of the role of mitochondrial K(ATP) channels and an overview of alternative mechanisms. *J Mol Cell Cardiol. 2005 May 19*

*79.* Erbel R, Heusch G. Coronary microembolization. *J Am Coll Cardiol 2000; 36:22-24*

*80.* Erbel R, Heusch G. Coronary microembolization - Its role in acute coronary syndromes and interventions. *Herz 1999; 24:558-575*

*81.* Grund F, Sommerschild HT, Lyberg T, Kirkeboen KA, Ilebekk A. Microembolization in pigs: effects on coronary blood flow and myocardial ischemic tolerance. *Am J Physiol 1999; 277:H533-H542*

*82.* Thielmann M, Dörge H, Martin C, Belosjorow S, Schwanke U, van de Sand A, Konietzka I, Büchert A, Krüger A, Schulz R, Heusch G. Myocardial dysfunction with coronary microembolization. Signal transduction through a sequence of nitroc oxide, tumor necrosis factor-alpha, and sphingosine. *Circ Res 2002; 90:807-813*

*83.* Schroder ES, Sirna SJ, Kieso RA, Kerber RE. Sensitization of reperfused myocardium to subsequent coronary flow reductions. An extension of the concept of myocardial stunning. *Circulation 1988; 78:717-728*

*84.* Schroder E, Kieso RA, Laughlin D, Schroder M, Meng R, Kerber RE. Altered response of reperfused myocardium to repeated coronary occlusion in dogs. *J Am Coll Cardiol 1987; 10:898-905*

*85.* Marti LSC, Pallares CV, Capdevila CC, Hernandiz MMA, Portoles SM, Cosin AJ. Ischaemic preconditioning. Is it always a beneficial phenomenon?. *Rev Esp Cardiol 1999; 52:429-436*

*86.* Mari C, Strauss WH. Detection and characterisation of hibernating myocardium. *Nucl Med Commun 2002; 23:311-322*

*87.* Bogaty P, Hackett D, Davies G, Maseri A. Vasoreactivity of the culprit lesion in unstable angina. *Circulation 1994; 90:5-11*

88. Napoli C, Liguori A, Chiariello M, Di Ieso N, Condorelli M, Ambrosio G. New-onset angina preceding acute myocardial infarction is associated with improved contractile recovery after thrombolysis. *Eur Heart J 1998; 19:411-419*
89. Pasceri V, Lanza GA, Patti G, Pedrotti P, Crea F, Maseri A. Preconditioning by transient myocardial ischemia confers protection against ischemia-induced ventricular arrhythmias in variant angina. *Circulation 1996; 94:1850-1856*
90. Vegh A, Szekeres L, Udvary E. Verapamil in a new model of severe local myocardial ischaemia due to combined coronary occlusion and stenosis. *Eur J Pharmacol. 1987; 144:15-27.*
91. Theroux P, Franklin D, Ross J, Kemper WS. Regional myocardial function during acute coronary artery occlusion and its modification by pharmacologic agents in the dog. *Circ Res 1974; 35:896-908*
92. Kowallik P, Schulz R, Guth BD, Schade A, Paffhausen W, Gross R, Heusch G. Measurement of regional myocardial blood flow with multiple coloured microspheres. *Circulation 1991; 83:974-982*
93. Kitakaze M, Node K, Minamino T, Asanuma H, Kuzuya T, Hori M. A Ca channel blocker, benidipine, increases coronary blood flow and attenuates the severity of myocardial ischemia via NO-dependent mechanisms in dogs. *J Am Coll Cardiol 1999; 33:242-249*
94. Koning MMG, Gho BCG, van Klaarwater E, Opstal RLJ, Duncker DJ, Verdouw PD. Rapid ventricular pacing produces myocardial protection by nonischemic activation of KATP+ channels. *Circulation 1996; 93:178-186*
95. Cohen MV, Downey JM. Myocardial stunning in dogs: preconditioning effect and influence of coronary collateral flow. *Am Heart J 1990; 120:282-291*
96. Noike H, Ohsava H, Uchi T, Kanai M, Yoshinuma M, Mineoka K, Hitsumoto T, Yokomuro H, Tokuhiro K, Tomioka H, Tachihara K. Effects of repetitive myocardial ischaemia on collateral circulation, ST deviation and epicardial wall motion. *J Cardiol 1997; 29:73-83*
97. Végh Á, Papp JG, Szekeres L, Parratt JR. The local intracoronary administration of methylene blue prevents the pronounced antiarrhythmic effect of ischaemic preconditioning. *Br J Pharmacol 1992; 107:910-911*
98. Végh Á, Papp JG, Parratt JR. Prevention by dexamethsaone of the marked antiarrhythmic effects of preconditioning induced 20 h after rapid cardiac pacing. *Br J Pharmacol 1994; 113:1081-1082*
99. Kis A, Vegh A, Papp JG, Parratt JR. Pacing-induced delayed protection against arrhytmias is attenuated by aminoguanidine, an inhibitor of nitric oxide synthase. *Br J Pharmacol 1999; 127(7):1545-50*
100. Kis A, Végh Á, Papp JG, Parratt JR. Cardiac pacing-induced delayed protection against ventricular arrhythmias: Evidence for the role of nitric oxide. *J ExpClin Cardiol 2000;5:17-24*
101. Kitakaze M, Node K, Minamino T, Asanuma H, Kuzuya T, Hori M. A Ca channel blocker, benidipine, increases coronary blood flow and attenuates the severity of myocardial ischemia via NO-dependent mechanisms in dogs. *J Am Coll Cardiol 1999; 33:242-249*
102. Kitakaze M, Node K, Minamino T, Kosaka H, Shinozaki Y, Mori H, Inoue M, Hori M, Kamada T. Role of nitric oxide in regulation of coronary blood flow during myocardial ischemia in dogs. *J Am Coll Cardiol 1996; 27:1804-1812*
103. Linz W, Wiemer G, Schölkens BA. Beneficial effects of bradykinin on myocardial energy metabolism and infarct size. *Am J Cardiol 1997; 80(3A):118A-123A*
104. Bernstein RD, Ochoa FY, Xu X, Forfia Pshen W, Thompson CI, Hintze TH. Function and production of nitric oxide in the coronary circulation of the conscious dog during exercise. *Circ Res 1996; 79:840-848*
105. Node K, Kitakaze M, Kosaka H, Komamura K, Minamino T, Tada M, Inoue M, Hori M, Kamada T. Plasma nitric oxide end products are increased in the ischemic heart. *Biochem Biophys Res Comm 1995; 211:370-374*

*106.* Node K, Kitakaze M, Kosaka H, Komamura K, Minamino T, Inoue M, Tada M, Hori M, Kamada T. Increased release of NO during ischaemia reduces myocardial contractility and improves metabolic dysfunction. *Circulation 1996; 93:356-364*

*107.* Zweier JL, Wang P, Kuppusamy P. Direct measurement of nitric oxide generation in the ischemic heart using electron paramagnetic resonance spectroscopy. *J Biol Chem 1995; 270:304-307*

*108.* Kitakaze M, Node K, Komamura K, Minamino T, Inoue M, Hori M, Kamada T. Evidence for nitric oxide generation in the cardiomyocytes: its augmentation by hypoxia. *J Mol Cell Cardiol 1995; 27:2149-2154*

*109.* Clementi E, Brown GC, Foxwell N, Moncada S. On the mechanism by which vascular endothelial cells regulate their oxygen consumption. *Proc Natl Acad sci USA 1999; 96:1559-1562*

*110.* Xu XP, Pollock JS, Tanner MA, Myers PR. Hypoxia activates nitric oxide synthase and stimulates nitric oxide production in porcine coronary resistance arteriolar endothelial cells. *Cardiovasc Res 1995; 30:841-847*

*111.* Muscari C, Bonafe' F, Gamberini C, Giordano E, Tantini B, Fattori M, Guarnieri C, Caldarera CM. Early preconditioning prevents the loss of endothelial nitric oxide synthase and enhances its activity in the ischemic/reperfused rat heart. *Life Sci. 2004; 74:1127-37*

*112.* Pan HL, Chen SR, Scicli GM, Carretero OA. Cardiac interstitial bradykinin release during ischemia is enhanced by ischemic preconditioning. *Am J Physiol 2000; 279:H116-H121*

*113.* Kis A, Yellon DM, Baxter GF. Role of nuclear factor-kappa B activation in acute ischaemia-reperfusion injury in myocardium. *Br J Pharmacol. 2003;138:894-900.*

*114.* Xuan YT, Tang XL, Banerjee S, Takano H, Li RCX, Han H, Qiu Y, Li JJ, Bolli R. Nuclear factor-{kappa}B plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. *Circ Res 1999; 84:1095-1109*

*115.* Bolli R, Dawn B, Tang XL, Qiu Y, Ping P, Xuan YT, Jones WK, Takano H, Guo Y, Zhang J. The nitric oxide hypothesis of late preconditioning. *Basic Res Cardiol 1998; 93:325-338*

*116.* Takano H, Manchikalapudi S, Tang XL, Qiu Y, Rizvi A, Jadoon AK, Zhang Q, Bolli R. Nitric oxide synthase is the mediator of late preconditioning against myocardial infarction in conscious rabbits. *Circulation 1998; 98:441-449*

*117.* Heusch G, Post H, Michel MC, Kelm M, Schulz R. Endogenous nitric oxide and myocardial adaptation to ischemia. *Circ Res 2000; 87:146-152*

*118.* Shah AM, MacCarthy PA. Paracrine and autocrine effects of nitric oxide on myocardial function. *Pharm Ther 2000; 86:49-86*

*119.* Node K, Kitakaze M, Sato H, Koretsune Y, Karita M, Kosaka H, Hori M. Increased release of nitric oxide in ischemic hearts after exercise in patients with effort angina. *J Am Coll Cardiol 1998; 32:63-68*

*120.* Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ. Relationship between cyclic guanosine 3':5'-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: effects of methylene blue and methemoglobin. *J Pharmacol Exp Ther 1981; 219:181-186*

*121.* Katsuki S, Arnold W, Mittal C, Murad F. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *Cyclic Nucleotide Res 1977; 3:23-35*

*122.* Shah AM, Spurgeon HA, Sollott SJ, Talo A, Lakatta EG. 8-bromo-cGMP reduces the myofilament response to Ca2+ in intact cardiac myocytes. *Circ Res 1994; 74:970-978*

*123.* Lohmann SM, Fischmeister R, Walter U. Signal transduction by cGMP in heart. *Basic Res Cardiol 1991; 86:503-514*

*124.* Xu KY, Huso DL, Dawson TM, Bredt DS, Becker LC. Nitric oxide synthase in cardiac sarcoplasmic reticulum. *Proc Natl Acad Sci USA 1999; 96:657-662*

*125.* Mery PF, Pavoine C, Belhassen L, Pecker F, Fischmeister R. Nitric oxide regulates cardiac Ca2+ current. Involvement of cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. *J Biol Chem 1993; 268:26286-95*

*126.* Xie YW, Shen W, Zhao G, Xu X, Wolin MS, Hintze TH. Role of endothelium-derived nitric oxide in the modulation of canine myocardial mitochondrial respiration in vitro. *Circ Res 1996; 79:381-387*

*127.* Brutsaert DL. Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhitmicity. *Psysiol Rev 2003; 83:59-115*

*128.* Suto N, Mikuniya A, Okubo T, Hanada H, Shinozaki N, Okumura K. Nitric oxide modulates cardiac contractility and oxygen consumption without changing contractile efficiency. *Am J Physiol 1998; 275:H41-H49*

*129.* Kelm M, Schaefer S, Dahmann R, Dolu B, Perings S, Decking UKM, Schrader J, Strauer BE. Nitric oxide induced contractile dysfunction is related to a reduction in myocardial energy generation. *Cardiovasc Res 1997; 36:185-194*

*130.* Brown GC. Nitric oxide regulates mitochondrial respiration and cell functions by inhibiting cytochrome oxidase. *FEBS Lett 1995; 369:136-139*

*131.* Horn R. The dual role of calcium: Pore blocker and modulator of gating. *Proc Natl Acad Sci 1999; 96:3331-3332*

*132.* Poderoso JJ, Peralta JG, Lisdero CL, Carreras MC, Radisic M, Schopfer F, Cadenas E, Boveris A. Nitric oxide regulates oxygen uptake and hydrogen peroxide release by the isolated beating rat heart. *Am J Physiol 1998; 274:C112-C119*

*133.* Ghafourifar P, Richter C. Nitric oxide synthase activity in mitochondria. *FEBS Lett 1997; 418:291-296*

*134.* Zhang X, Xie YW, Nasjletti A, Xu X, Wolin MS, Hintze TH. ACE inhibitors promote nitric oxide accumulation to modulate myocardial oxygen consumption. *Circulation 1997; 95:176-182*

*135.* Sommerschild HT, Lunde PK, Deindl E, Jynge P, Ilebekk A, Kirkeboen KA. Elevated levels of endogenous adenosine alter metabolism and enhance reduction in contractile function during low-flow ischemia: Associated changes in expression of Ca2+-ATPase and phospholamban. *J Mol Cell Cardiol 1999; 31:1897-911*

*136.* Domenech R, Macho P, Schwarze H, Sanchez G. Exercise induces early and late myocardial preconditioning in dogs. *Cardiovasc Res 2002; 55:561-566*

*137.* Abete P, Ferrara N, Cacciatore F, Sagnelli E, Manzi M, Carnovale V, Calabrese C, de Santis D, Testa G, Longobardi G, Napoli C, Rengo F. High level of physical activity preserves the cardioprotective effect of preinfarction angina in elderly patients. *J Am Coll Cardiol 2001; 38:1357-65*

*138.* Lambiase PD, Edwards RJ, Cusack MR, Bucknall CA, Redwood SR, Marber MS. Exercise-induced ischemia initiates the second window of protection in humans independent of collateral recruitment. *J Am Coll Cardiol 2003; 41:1174-1182*

*139.* Bilinska M, Rudnicki S, Beresewicz A. Delayed attenuation of myocardial ischemia with repeated exercise in subjects with stable angina: a possible model for the second window of protection?. *Basic Res Cardiol 2000; 95:418-423*

*140.* Végh Á. Exercise-induced delayed preconditioning in the protection of the heart against arrhythmias. *Cardiologia Hungarica 2003; 33:187-196*

*141.* Sessa WC, Pritchard K, Seyedi N, Wang J, Hintze TH. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ Res 1994; 74:349-353*

142. Kingwell BA. Nitric oxide as a metabolic regulator during exercise: Effect of training in health and disease. Clin Exp Pharmacol Physiol 2000; 27:239-250

*143.* Heberden J. Letter to the editor. Medical transactions, *College of Physicians London 1785; 3:1-11*

*144.* Pfaffenbarger RS, Hale WE, Brand RJ, et al. Work-energy level, personal characteristics, and fatal heart attack: a birthcohort effect. *Am J Epidemiol 1997; 105:200-213*

*145.* Siscovick DS, Weiss NS, Hallstrom AP, et al. Physical activity and primary cardiac arrest. *JAMA 1982; 248:3113-3117*

*146.* Abete P, Calabrese C, Ferrara N, Cioppa A, Pisanelli P, Cacciatore F, Longobardi G, Napoli C, Rengo F. Exercise training restores ischemic preconditioning in the aging heart. *J Am Coll Cardiol 2000; 36:643-650*

*147.* Kay IP, Kittelson J, Stewart RAH. Collateral recruitment and "warm-up" after first exercise in ischemic heart disease. *Am Heart J 2000; 140:121-125*

*148.* Okazaki Y, Kodama K, Sato H, Kitakaze M, Hirayama A, Mishima M, Hori M, Inoue M. Attenuation of increased regional myocardial oxygen consumption during exercise as a major cause of warm-up phenomenon. *J Am Coll Cardiol 1993; 21:1597-1604*

*149.* Rinaldi CA, Masani ND, Linka AZ, Hall RJ. Effect of repetitive episodes of exercise induced myocardial ischaemia on left ventricular function in patients with chronic stable angina: evidence for cumulative stunning or ischaemic preconditioning? *Heart 1999; 81:404-411*

*150.* Edwards RJ, Redwood SR, Lambiase PD, Tomset E, Rakhit RD, Marber MS. Antiarrhythmic and anti-ischaemic effects of angina in patients with and without coronary collaterals. *Heart 2002; 88:604-610*

*151.* Williams DO, Bass TA, Gewirtz H, et al. Adaptation to the stress of tachycardia in patients with coronary artery disease: insight into the mechanism of the warm-up phenomenon. *Circulation 1985; 71:687-692*

*152.* Thadani U, Lewis JR, Mathew TM, et al. Reproducibility of clinical and haemodynamic parameters during pacing stress testing in patients with angina pectoris. *Circulation 1979; 60:1036-1044*

*153.* Homans DC, Sublett E, Dai XZ, et al. Persistance of regional left ventricular dysfunction after exercise-induced myocardial ischaemia. *J Clin Invest 1986; 77:66-73*

*154.* Bogaty P, Kingma JG, Robitaille NM, Plante S, Simard S, Charbonneau L, Dumesnil JG. Attenuation of myocardial ischemia with repeated exercise in subjects with chronic stable angina. Relation to myocardial contractility, intensity of exercise and the adenosine triphosphate-sensitive potassium channel. *J Am Coll Cardiol 1998; 32:1665-1671*

*155.* Kelion AD, Webb TP, Gardner MA, Ormerod OJM, Banning AP. The warm-up effect protects against ischemic left ventricular dysfunction in patients with angina. *J Am Coll Cardiol 2001; 37:705-710*

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## 7. ANNEX

### Reprints of full papers

I. *Clinical Science 2002; 102:435-445*

II. *Clinical Science 2004; 106: 201-213*

III. *Cardiologia Hungarica 2004; 34:241-248*

### Manuscript of the book chapter

IV. *In: Myocardial ischaemia and preconditioning. Kluwer Academic Publishers, Boston; 2003: pp. 423-443*

### Manuscripts of the quotable abstracts

V. *Journal of Molecular and Cellular Cardiology 1999; 31: A97*

VI. *Cardiologia Hungarica 1999; Suppl. 2: 57*

VII. *Zeitschrift für Kardiologie 2000; Suppl. 5*

VIII. *Cardiologia Hungarica 2000; Suppl. 3: 24*

IX. *Perfusion 2000; 15: Abstract Suppl.*

X. *Cardiologia Hungarica 2001; Suppl. 2: 26*

XI. *Acta Clinica Croatica 2001; Suppl. 1: O27*

XII. *European Heart Journal 2001; Abstract Suppl.: P3629*

XIII. *Journal of Molecular and Cellular Cardiology 2002;34: A62*